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FISHERIES RESEARCH BOARD OF CANADA

OTTAWA, 1961

1960

STUDIES

FROM THE FISHERIES RESEARCH BOARD OF CANADA

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CANADA
DEPARTMENT OF NORTHERN AFFAIRS AND NATIONAL RESOURCES

THE FAUNA OF MINAS BASIN AND MINAS CHANNEL

By
E. L. Bousfield
and
A. H. Leim

(From National Museum of Canada, Bulletin No. 166, Contributions to Zoology, 1958)

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THE FAUNA OF MINAS BASIN AND MINAS CHANNEL

By E. L. Bousfield and A. H. Leim[1]

Introduction

Minas Channel and Basin, including Cobequid Bay, at the upper end of the Bay of Fundy, form an unusual coastal marine habitat. The tidal amplitude of up to 53 feet is unique; it results in very thoroughly mixed water, which, because it is relatively shallow, is warmer in summer and colder in winter than that of the Bay of Fundy proper. This strong tidal mixing keeps much silt in suspension in the Basin, making for extreme turbidity and relatively little penetration of light into the surface waters. The intertidal zone is remarkable not only for its extreme vertical range, but also for its extreme horizontal range, because of gentle shore-line gradients; some beaches are more than three miles wide at low water. Reflecting these unusual physical conditions is the remarkable composition of the fish and bottom fauna. A detailed listing of the more conspicuous marine animals of the region and an inquiry into the relationship between their distribution and environments are the chief purpose of this report.

One of the authors (A.H.L.) collected material at a few points in this area in the summers of 1920 to 1922 while engaged in a study of the shad (*see* Leim, 1924). The other (E.L.B.) collected somewhat more widely during the summer of 1958. The two investigations thus provide a reasonably comprehensive picture of the marine macro-fauna of the region and afford a comparison over a period of some thirty-five years.

The early collections were made at Scotsman Bay, Nova Scotia, in August of 1920 and 1922 and at Bass River and Tennycape, N.S., in August and early September, 1921. Weir catches provided most of the fish; the invertebrates were mainly from shore collections. The Atlantic Biological Station boat *E.E. Prince* carried out hydrographical measurements and biological collecting at seven stations, numbers 190 to 196, during the first ten days of September, 1920. These stations were in Minas Channel and the lower part of Minas Basin (*see* Fig. 1). The hydrographical and biological results are given in Table 2 and in the main body of this report, respectively.

During the period June 23 to August 2, 1958, some thirty stations along the shores of the region were visited. Collections of intertidal invertebrates were made at sixteen stations, and hydrographical measurements at seven (*see* Table 1 and Fig. 1). More than 130 species of littoral marine invertebrates were obtained, of which the crustaceans and molluscs, making up 90 per cent of the total, are treated herein. Results of brief collecting trips to Economy Point, Bass River, Tennycape, and Walton, in June, 1950, are also included.

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80553-1—1½

Acknowledgments

The early studies were carried out under the auspices of the Biological Board of Canada and the University of Toronto. Acknowledgment for assistance in the identification of various invertebrate groups is gratefully made to specialists concerned, whose names are given in the station lists of material. The recent field work was carried out under the National Museum of Canada in co-operation with the Fisheries Research Board of Canada. Special mention is made of Dr. A. G. Huntsman for much advisory assistance and for the identification of Ascidians and Chaetognatha.

Physical Characteristics of the Region

The region under investigation lies in the north-central part of Nova Scotia (fig. 1). Minas Channel is an S-shaped sluiceway about 30 miles in length, linking Minas Basin with the Bay of Fundy. It is 15 miles wide at the outer end in a line from Cape Chignecto to Harbourville (S13), and narrows to about 4 miles at the inner end between Cape Blomidon and Partridge Island, near Parrsboro. The total area of the Channel is 300 square miles (planimeter measurement). Because the shores are typically steep and rocky, the total intertidal area is only 5 square miles. Indentations at Advocate, Spencer's Island, Scotsman Bay, and West Bay are characterized by a hard shelving foreshore of sand and mud, usually bordered at the HW line by coarse gravel. During relatively quiet summer months, several inches of soft mud may accumulate on top of the sand but is washed away during winter storms.

Most of the Channel is between 25 and 50 meters in depth, ranging down to 100 meters in the central trough. The mean high water level in Minas Channel is approximately 10.7 meters (35 feet) above 0 datum, and the mean tidal difference is 9.1 meters (30 feet). The mean HW depth is 46 meters (126 feet) and the LW depth (0 datum) is 35.3 meters (97 feet). The speed of tidal currents at all depths in the narrow part of the Channel reaches six to eight knots. Although carried back and forth by tidal action, the water to the south of the main channel seems to be stationary over a complete tidal cycle. What is locally known as the "cedar swamp" occurs here, a miniature Sargasso Sea, in which seaweed, wreckage, and other debris collect. The material drifts back and forth with the tidal ebb and flow from near Cape Split at the inner end to a point south of Isle au Haute at the outer. Local fishermen state that this debris remains in the region until it becomes water-logged and sinks. They cite, as evidence, the case of a small wrecked vessel which drifted in this locality for months.

Minas Basin and Cobequid Bay form a triangular body of water 50 miles long and 18 miles wide at its western base. The total surface area (including estuaries) of 435 square miles is nearly half again that of the Channel. Most of the Basin is less than 25 meters in depth, except for a deeper tongue intruding from Minas Channel near the mouth. The mean depth at LW (0 datum) is 14.6 meters (48 feet). The mean rise of tide above datum is approximately 13.8 meters (45 feet), the mean intertidal difference is 11.9 meters (39 feet), and the mean depth of the Basin at HW is 20.9 meters (68.5 feet).

Ketchum *et al.* (1953) have given values for the intertidal and high-tide volumes and exchange ratios of various tidal exchange segments in Minas Channel and Basin. Thus, during ebb tide, water at the mouth of Cobequid Bay moves to a point near the mouth of the Basin. The exchange ratio in this segment (intertidal : high tide volume) is 0.6; in other words, about 60 per cent of the water mixes with the next seaward segment and does not return on the flood. The exchange ratio is extremely high in Cobequid Bay (76 to 94 per cent) but is much lower in Minas Channel (23 to 39 per cent) because of the smaller tidal amplitude and greater depth.

The intertidal zone of the entire Basin is mainly sandy mud overlying Carboniferous and Triassic sandstone. During summer, soft mud collects on the tidal flats, especially in the estuaries, to a depth of 8 to 12 inches. The entire eastern half of Cobequid Bay and the mouth of the Avon estuary is sandy mud. At extreme low water the area of the intertidal region is approximately 153 square miles, or more than one-third the total area of the Basin. No other coastal marine area in the world of comparable size has such a large proportion of bottom exposed to the air environment at low tide.

The amount of fresh water entering Minas Basin and Channel, particularly from rivers of the south side, is appreciable and supplies about 15 per cent of the total mean annual flow into the Bay of Fundy. The total area of the Minas watersheds is 3,300 square miles (Watson, 1936), or about four times the surface area of Minas Basin and Channel combined (735 square miles). During the summer months (July to Sept.) an average value of about 3,500 c.f.s.* is discharged into the Basin and Channel, more than half from the Avon and Shubenacadie watersheds. Since, however, the total drainage area relative to that of the Basin and Channel is small (approx. 4:1), the effect of the fresh water on surface salinities is likewise small. Moreover, owing to vigorous tidal mixing, surface salinities, except at the head of Cobequid Bay and in the southern bight, are typically marine rather than estuarine in character.

Hydrographic Conditions

A striking feature of the hydrography of the region is the pronounced vertical mixing by tidal action. The results of the *Prince* expedition in the area in early September, 1920, show near-uniform temperatures (14.0-14.4°C. and salinities (31.26-31.4‰) both vertically (0-70 meters) and horizontally across Minas Channel to Scotsman Bay (Table 2). Also, at the mouth of the Basin (*Prince* 194) near-uniform temperatures (14.35-14.5°C.) and salinities (31.29-31.33‰) prevailed from surface to 45 meters. However, progressing southward into the Basin, we note significantly higher temperatures and lower salinities at all depths, and greater vertical differences, reaching 16.5°C. and 30.84‰ respectively at the surface at *Prince* 196, northeast of Kingsport. Southward extrapolation of this trend would indicate that the southern bight of Minas Basin is distinctly more estuarine in physical characteristics than the main part of the Basin.

* This is equivalent to an average summer value of about 1.0 c.f.s. per square mile of drainage area, which compares favourably with values calculated for the Miramichi watershed in northern New Brunswick (c.f. Bousfield, 1955a).

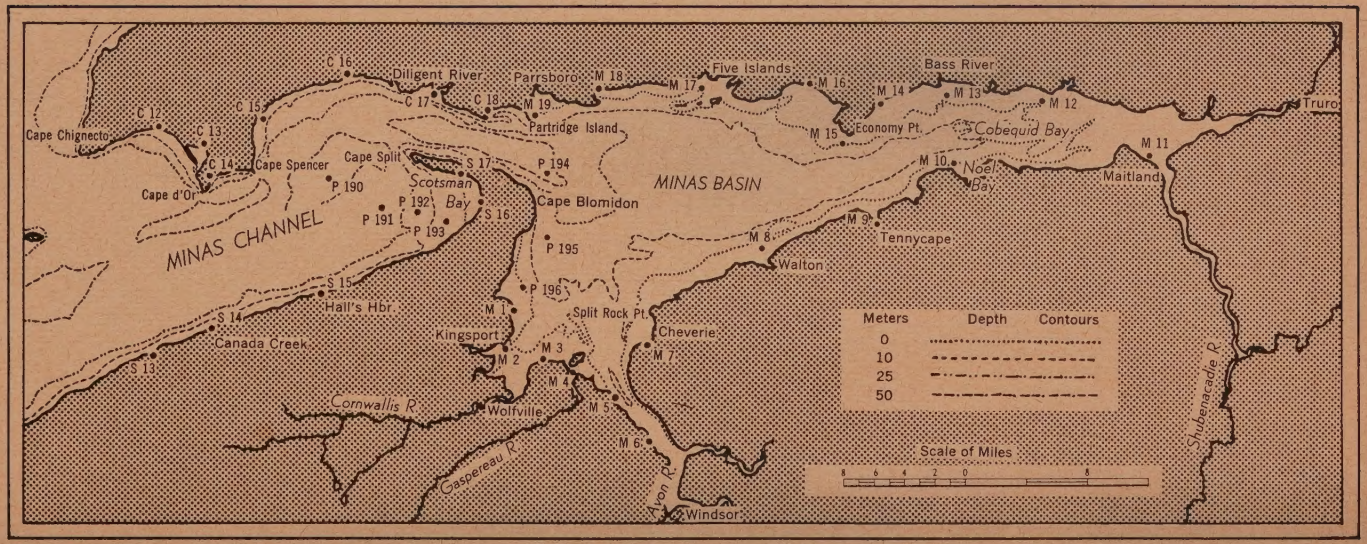

Figure 1. Localities visited in the Minas Channel – Minas Basin region, 1920-22, 1950 and 1958.

Table 1. Stations Visited

Key to Sta. numbers: S —South coast of Minas Channel
M—Minas Basin
C—Chignecto coast of Minas Channel

Sta. No.	Date (1958)	Locality	Habitat	Water Temp. (°C.)	Water Sal'y. (‰)
		Kings Co.—			
S14	June 27	Canada Creek	Boulders, stones, and gravel; LW-HW levels.	11.9	30.8
S15	" 27	Hall's Harbour	Gravelly mud flats of river mouth; rock pools; MW-HW levels.	12.0	—
S16	" 24	Scotsman Bay	Coarse gravel beach, HW level.	—	—
S17	" 24	Scotsman Bay	Rock pools near HW level.	18 (tide pool)	—
M1	" 25	Shore opp. Paddy I.	Sandstone cliffs, sandy mud flats; MW-HW levels.	—	—
M2	" 25	Kingsport	Sand and sandy mud flats HW-LW levels.	22	—
M3	" 26	Evangeline Beach	Coarse sand; mud and boulders; HW 1 level.	—	—
M4	" 26	Avonport Beach	Coarse sand, sandstone and mud; MW-HW levels.	—	—
M5	" 27	Blue Beach	Shingle and pebble beach HW level	—	—
		Hants Co.—			
M6	" 27	Aberdeen Beach	Coarse sand and mud, HW level	—	—
M7	July 17	Cheverie wharf	Coarse sand; HW level	—	—
M8	" 17	Walton	Shale, mud, sand; LW-HW levels	17.5	—
M9	" 18	Tennycape Hbr.	Sand spit; HW 1 level	—	—
M10	" 18	Burntcoat Hd., at Noel Bay	Sandstone; stones, gravel, muddy sand. LW-HW levels	17.2	29.3
M11	" 19	Maitland, N. of wharf	Mud, sand, sandstone LW-MW levels	19.8	24.6
		Colchester Co.—			
M12	" 20	Spencer Pt.	Muddy sand, sandstone; LW	18.5	27.8
M13	" 20	Bass R., at fish weir	Pebbles, small stones, sandy mud; LW-HW levels	—	—
M14	" 21	Cove Road Beach, Economy Pt.	Steep gravel beach; MW-HW levels	—	—
M15	" 21	Economy Pt.	Sandstone boulders, sand and shells; LW-HW levels	—	—
M16	Aug. 2	Lower Economy at wharf	Coarse sand and gravel, at HW level	—	—
M17	" 2	Lower Five Islands at at wharf and weirs	Mud, sandy mud, gravel; LW-HW levels	18.0	29.6
		Cumberland Co.—			
M18	July 22	Mouth of Swan Creek, nr. Clarke Hd.	Coarse sand and gravel at HW level	—	—
M19	" 22	Partridge I., south of Parrsboro	Rock, boulders, sand and gravel; LW-HW levels	14.2	30.7
C18	" 22	West Bay	Coarse gravel beach at HW	—	—
C17	" 23	Diligent R.	Gravel and sand; LW-HW levels.	14.2	—
C16	Aug. 1	Port Greville	Course gravel beach at HW	—	—
C15	" 2	Spencer's Island	Fine sand, gravel, boulders. LW level	13.0	30.9
C14	July 31	Horseshoe Cove, nr. Cape d'Or	Coarse gravel and sand at HW	—	—
C13	" 31	Advocate Harbour	Sandy mud, stones; nr. HW	—	—
C12	" 31	West Advocate	Gravel beach and sandstone HW level	—	—

Figure 2. Surface temperatures (°C.) in the Minas Basin region, LW values, June 25 to Aug. 2, 1958.

Table 2.

Hydrographical results of the "Prince" Expedition in Minas Channel and Basin, September, 1920

Minas Channel, Sept. 4/1920												Minas Basin, Sept. 8/1920								
P 190 8:10 a.m.			P 191 6:45 a.m.			P 192 9:55 a.m.			P 193 10:35 a.m.			P 194 8:45 a.m.			P 195 10:17 a.m.			P 196 12:36 p.m.		
D. (m)	Temp. (°C.)	Sal'y. (°/₀₀)	D. (m)	Temp. (°C.)	Sal'y. (°/₀₀)	D. (m)	Temp. (°C.)	Sal'y. (°/₀₀)	D. (m)	Temp. (°C.)	Sal'y. (°/₀₀)	D. (m)	Temp. (°C.)	Sal'y. (°/₀₀)	D. (m)	Temp. (°C.)	Sal'y. (°/₀₀)	D. (m)	Temp. (°C.)	Sal'y. (°/₀₀)
0	14.25	31.26	0	14.0	31.27	0	14.4	31.27	0	14.4	31.29	0	14.5	31.29	0	15.0	31.11	0	16.47	30.84
10	"	31.27	10	14.1	31.40	12	14.1	31.29	4	14.3	31.29	10	14.37	31.29	10	14.8	31.17	6	15.56	30.84
25	"	31.27	25	14.08	31.33			—		—	—	25	14.35	31.33	20	14.75	31.17	—	—	—
50	"	31.29	—	—	—	—	—	—	—	—	—	45	14.35	31.33	—	—	—	—	—	—
70	"	31.31	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

The observations of the *Prince* are, in general, corroborated by measurements of temperature and salinity made at low water level at selected localities around the shores of the region during early summer of 1958 (fig. 2 and 3). Fig. 2 indicates that surface temperatures in the central Basin were between 15° and 18°C., whereas those of the southern Bight and Cobequid Bay were consistently above 17°C., reaching more than 20°C. in the Avon and Shubenacadie estuaries respectively. Here the surface waters are heated as they roll in over the extensive tidal flats that are warmed by the sun when the tide is out.

Fig. 3 suggests more or less uniformly high surface salinities (29.5-30.5‰) over the main Basin, decreasing rather sharply through the southern bight and Cobequid Bay to values of less than 25‰ in the Avon and Shubenacadie estuaries respectively. On the average, the numerical values of salinity for 1958 are about 0.5‰ lower than those at corresponding localities in 1920 owing to the fact that the former measurements were made (1) at dead low water or nearly so and thus minimal for the tidal cycle, (2) at hip-boot distance from shore and presumably under slight influence of fresh water seepage from the land, and (3) during early summer (June and July) when the effect of spring freshets was still being felt. A value of 29.01‰ taken at Bass River on August 15, 1921, is correspondingly higher than the 1958 value of about 28.5‰ predicted from fig. 3.

The extreme turbidity of the water in this region is indicated by Secchi disc values given by Huntsman (1952). The values decreased from 5.75 to 10.25 meters (20 to 35 feet) in the Bay of Fundy, through 3.00 to 3.75 meters (10 to 12 feet) in Minas Channel, down to 1.25 meters (4 feet) off Kingsport in the Basin. Shore observations in 1950 and 1958 indicate that values in the southern bight and throughout Cobequid Bay are still lower and probably average less than 0.5 meters (1.5 feet). At some collecting localities, when the hands were placed wrist-deep in the water the fingertips were not visible.

History of the Sea Fisheries of the Region

Although several writers have referred to the shad and salmon of these waters, very few have given any account of the fishing methods employed. Perley (1852) seems to have given about the only information that is available. This following account is derived mainly from that source and from the evidence given before the Dominion Shad Fishery Commission (Prince, *et al.*, 1910).

The most important fishery of the area from early times seems to have been the shad. Two main methods have been employed. First, weirs either of brush or of twine ("seines") were used. These were often of great length and captured large numbers of shad and some salmon. On the north shore of Minas Basin there was an average of one weir for every mile of shore, and "between Graham's Head and Economy Point, the flats for four miles were observed to have an almost unbroken continuance of these weirs, crescent-shaped, the ends of the weirs touching each other" (Perley).

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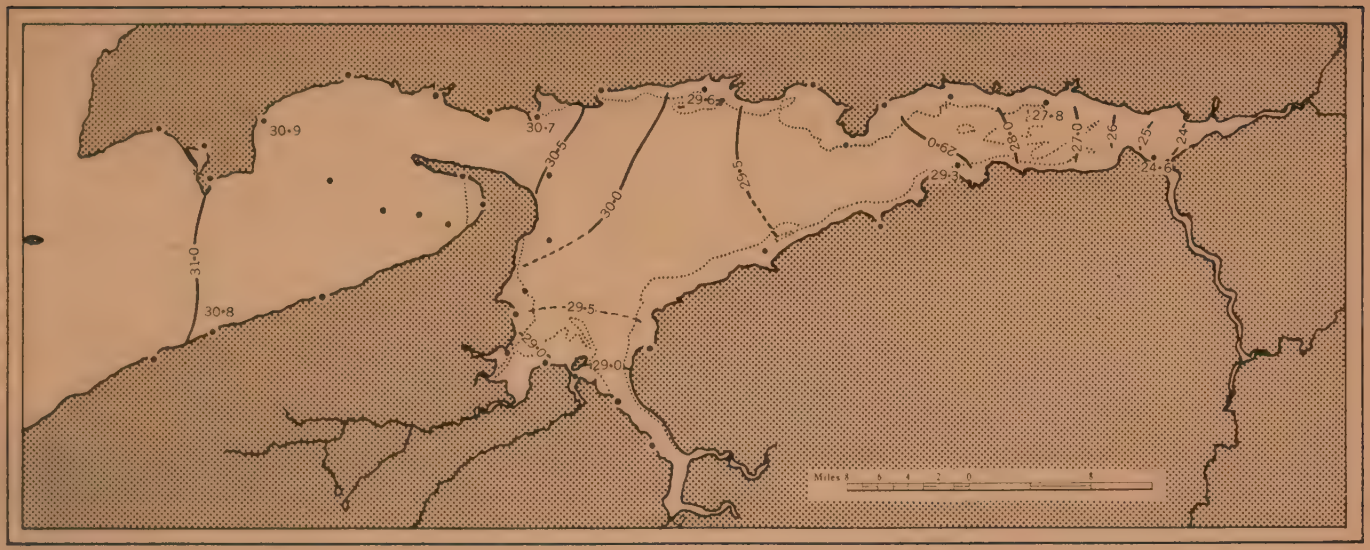

Figure 3. Surface salinities (‰) in the Minas Basin region, LW values, June 25 to Aug. 2, 1958.

Drift nets were also used in the shad and salmon fisheries. Using this method the boats put out several hundred yards of gill net in the upper part of the Basin and drifted down the ebb tide and back to the starting point with the flood. It is stated that the use of drift nets began about 1840 on the Windsor (Avon) River.

The drift net gave rise to another method of fishing, which is known as a "stake net" or "set net". Instead of allowing the nets to drift freely, they were attached to stakes driven in the mud of the intertidal zone. These were unlike the "seine" weirs in that they were set in a straight line; one is recorded at Burntcoat Head as being 3 miles long, and the fish were caught in the meshes. Shad fishing was carried on in Scotsman Bay, Minas Basin, and Cobequid Bay but not in Minas Channel.

The salmon fishery was the next most important and was carried on in the same way as the shad, that is by means of brush weirs and drift nets. Salmon were obtained in the largest quantities in the Avon River, Cobequid Bay, and Economy River. More recently, Huntsman (1958) has dealt with fluctuations in the population of salmon in the Shubenacadie estuary and Cobequid Bay.

The other fisheries were minor ones. Although gaspereau were abundant, and many were caught in weirs, there appeared to be no demand for this fish.

Some of the weirs in Minas Channel obtained fat spawning herring, and this was apparently an important fishery in a localized area. It was stated that "they deposit such quantities of spawn that it can be shovelled up upon the beach."

There was fishing for cod in Minas Channel on the north shore, from Parrsboro to Cobequid Bay, and at the mouth of the Avon. This fishery was a primitive one. Poles were driven into the flats at low tide and baited hooks attached. On the following low tide the fish were collected.

Good pollock and some haddock fishing is reported on the north shore of Minas Channel as far as Cape Sharp. Some halibut were obtained in the same locality, and large quantities of flounder were said to have been smoked at West Bay, just east of Cape Sharp.

In addition, "tons of eels," many smelt, and some large sturgeon were taken to the south of Cape Blomidon.

Some bass were obtained farther up the Basin, but even in 1850 this fishery had become an unimportant one owing to the small quantities available.

The methods of fishing described by Perley are being employed today with scarcely any change. Owing to the decline of the shad and salmon, however, there has been a very great reduction in the number of weirs and drift-net boats, and except for herring nets on the south shore opposite Isle au Haute, it is doubtful if stake nets have been set. Cod, with the exception of those taken in the weirs, are still fished by means of lines attached to stakes and set out on the flats. The cod and herring do not seem to have been included in the decline which occurred in most of the other fisheries.

Previous Collections in the Region

Surprisingly little has been published on the marine fauna of the Minas Basin region. Whiteaves (1901) summarized the 19th century invertebrate records and listed only six species. Two of these were sponges (*Chalina oculata* Pallas and *Desmacidon palmata* (Johnston)) collected by C. W. Willmot at Five Islands in 1882, and four were molluscs (*Volsella demissus* (Dillwyn), *Astarte undata* Gould, *A. castanea* Say, and *Crepidula fornicata* L.) taken by G. T. Kennedy at various localities in 1875. Kindle (1916, 1917) reported on certain collections, mainly intertidal, which he and Whittaker made at Kingsport, near *Prince* 196, and at Cheverie Pt., on the eastern side of the mouth of the Avon estuary. The material included one species of sponge, one of annelids, five of bryozoans, twenty-nine of molluscs, two of echinoderms, and four of crustaceans. Between 1922 and the present, published references to selected invertebrates of the Minas Basin region have been made by Huntsman (1924), Johansen (1930), Bousfield (1952), and again by Huntsman (1952), this last being a comprehensive analysis of the recent status of the marine fisheries with reference to food organisms, both benthonic and planktonic, and to factors underlying the marine productivity of the basin. The Stephensons (1954) carried out a detailed ecological survey of the intertidal region at Hall's Harbour and described the vertical range of conspicuous invertebrate animals and marine algae. Finally Jermolajev (1958) described the zooplankton of the region, noting the abundance of such endemic species as *Acartia tonsa* Dana, *Eurytemora herdmani* Thompson and Scott, *Pseudodiaptomus coronatus* Williams, and a form of *Centropages hamatus* (Lillj.).

Faunal List

I. Fishes (Phylum CHORDATA; Class Pisces)

Alopias vulpinus (Bonnaterre). Thresher shark. Not seen by the author, but reliably reported from Scotsman Bay and Parrsboro.

Squalus acanthias Linnaeus. Spiny dogfish. Occasional in weir at Scotsman Bay. More abundant at *Prince* station 191.

Raja laevis Mitchill. Barndoor skate. Occasional throughout the region.

Raja diaphanes Mitchill. Big skate. Occurs throughout the region.

Raja erinacea Mitchill. Tobacco box skate. Abundant throughout the region.

Acipenser sturio Linnaeus. Sturgeon. Taken occasionally. A few are caught annually in the Avon River.

Clupea harengus Linnaeus. Herring. Common throughout the region, all sizes being represented. Spawning occurred, and fry were caught in plankton tows in Minas Channel and Basin, and at Bass River.

Pomolobus pseudoharengus (Wilson). and/or

Pomolobus aestivalis (Mitchill) Alewife. Immature specimens from 9 to 29 cm long were abundant at Bass River and Scotsman Bay. Mature fish ascend the Avon and Shubenacadie Rivers at spawning time. Whether all belong to the former species is in doubt.

Alosa sapidissima (Wilson). Shad. Specimens of all sizes from 17 to 60 cm in length were abundant at Scotsman Bay and Bass River. They spawn in the Shubenacadie River.

Salmo salar Linnaeus. Atlantic salmon. Taken at Bass River and Scotsman Bay in weirs, and in drift nets and traps throughout the Channel and Basin. There is a late summer run of spawning fish into the Shubenacadie River.

Osmerus mordax (Mitchill). Smelt. Mature and immature smelt occur throughout the area. They ascend the tributary rivers and streams at spawning time.

Anguilla rostrata (LeSueur). Eel. Moderately large specimens, up to 110 cm long, taken in weirs at Scotsman Bay and Bass River.

Fundulus heteroclitus (Linnaeus). Mummichog. Taken occasionally at Bass River and in the estuary of the Shubenacadie River.

Merluccius bilinearis (Mitchill). Silver hake. Very abundant in late summer at Scotsman Bay; less frequent at Bass River.

Gadus callarias Linnaeus. Cod. Infrequent in summer at Scotsman Bay and absent at Bass River. Abundant in both places in spring and autumn, when they are fished for.

Microgadus tomcod (Walbaum). Tomcod. Mature specimens not abundant but immature stages very abundant at Bass River, less so at Scotsman Bay. Spawns in the Shubenacadie River, where the fry are numerous in the estuary.

Pollachius virens (Linnaeus). Pollock. Infrequent at Scotsman Bay.

Urophycis tenuis (Mitchill). and/or

Urophycis chuss (Walbaum). Hake. Mature hake were abundant at *Prince* station 191 and smaller numbers were caught at stations 192-196. Immature sizes were abundant in the weirs at Scotsman Bay and Bass River.

Pseudopleuronectes americanus (Walbaum). Winter flounder. Moderately common at Scotsman Bay, Bass River, and at *Prince* stations 191-3, 195-6. Those caught at Bass River were smaller. Larvae were found at Bass River and at *Prince* stations 191 and 195.

Liopsetta putnami (Gill). Smooth flounder. Infrequent at Scotsman Bay; it is the most abundant flounder at Bass River.

Lophopsetta maculata (Mitchill). Sand flounder. Common at Scotsman Bay and Bass River.

Menidia menidia (Linnaeus). Silverside. Very abundant at Bass River and in the estuary of the Shubenacadie River. Taken once near Cape Blomidon.

Syngnathus fuscus Storer. Pipe-fish. Occasional at Bass River.

Scomber scombrus Linnaeus. Mackerel. An irregular visitor at Scotsman Bay; mature fish are rare, but "tinkers," 23 to 33 cm long, sometimes appear in quantity.

Poronotus triacanthus (Peck). Dollar-fish. Immature specimens are common at Scotsman Bay and Bass River and are caught in drift nets throughout Minas Basin.

Roccus saxatilis (Walbaum). Striped bass. All sizes, from 5 cm in length up, were present at Bass River; adults were infrequent, but the smaller sizes were very abundant. This species spawns in the Shubenacadie River, where its larvae are very numerous in July.

Myoxocephalus aeneus (Mitchill). Grubby. Taken occasionally at Scotsman Bay and at *Prince* stations 195 and 196.

Myoxocephalus octodecimspinosus (Mitchill). Long-horn sculpin. Occasional at Scotsman Bay and at *Prince* station 192.

Hemitripterus americanus (Gmelin). Sea raven. Common at Scotsman Bay. Young specimens taken at *Prince* stations 192 and 195.

Cyclopterus lumpus Linnaeus. Lumpfish. One small specimen was seen at Scotsman Bay.

Ammodytes americanus DeKay. Sand launce. Infrequent at Bass River.

Pholis gunnellus (Linnaeus). Rock eel. Found near Cape Blomidon and Cape Split and at *Prince* station 195.

Macrozoarces americanus (Bloch and Schneider). Eel pout. Found at Scotsman Bay; young individuals occurred at *Prince* stations 191, 192, and 195.

Lophius americanus Cuvier and Valenciennes. Angler. Frèquent at Scotsman Bay.

COLENTERATA: HYDROMEDUSAE

(Identifications of medusoids by Dr. H. B. Bigelow, Museum of Comparative Zoology, and of hydroids by Professor C. M. Fraser, University of British Columbia.)

I. ANTHOMEDUSAE (medusoid stage)

Podocoryne carnea Sars. Found at *Prince* stations 195 and 196 and at Bass River.

Nemopsis bachei L. Agassiz. Found at Bass River. Not previously reported north of Cape Cod.

II. ANTHOMEDUSAE (hydroid or non-medusoid stage)

Acaulis primarius (Stimpson). At *Prince* stations 191 and 193.

Corymorpha pendula Agassiz. At *Prince* station 195.

Eudendrium tenue Agassiz. At *Prince* stations 190, 195, and 196.

Hydractinia echinata (Fleming). At Scotsman Bay, intertidal.

Tubularia spectabilis (Agassiz). At *Prince* stations 191 and 195.

Tubularia crocea (Agassiz). At *Prince* station 195.

III. Leptomedusae (hydroid stage)

Campanularia verticillata (Linnaeus). At *Prince* stations 194 and 196.

Gonothyraea loveni (Allman). At *Prince* station 196.

Obelia dichotoma (Linnaeus). At *Prince* station 196.

Obelia geniculata (Linnaeus). At *Prince* station 195; intertidal at Scotsman Bay and Bass River.

Calycella syringa (Linnaeus). At *Prince* stations 194 to 196.

Halecium beani (Johnston). At *Prince* stations 194 to 196; intertidal at Bass River.

Halecium halecinum (Linnaeus). At *Prince* station 196; intertidal at Scotsman Bay.

Abietinaria abietina (Linnaeus). At *Prince* station 196.

Diphasia fallax (Johnston). At *Prince* stations 190, 192, 194 to 196; intertidal at Bass River.

Diphasia rosacea (Linnaeus). At *Prince* stations 195, 196; intertidal at Bass River.

Hydrallmania falcata (Linnaeus). At *Prince* stations 190, 194 to 196; intertidal at Bass River.

Sertularella rugosa (Linnaeus). At *Prince* stations 195.

Sertularella tricuspidata (Alder). At *Prince* stations 192, 195, 196; intertidal at Bass River.

Thuiaria cupressina (Linnaeus). At *Prince* stations 194 to 196; intertidal at Bass River.

Thuiaria similis (Clark). At *Prince* station 196.

Antennularia americana Nutting. At *Prince* station 192.

Antennularia antennina (Linnaeus). At *Prince* stations 195, 196.

ANNULATA:CHAETOPODA

(Identifications by Professor A. L. Treadwell, Vassar College.)

I. Polychaeta:Nereidiformia

Autolytus sp. Taken in tows at Scotsman Bay and Bass River.

Lepidonotus squamatus (Linnaeus). At Prince stations 190, 192, 195, and 196, being abundant at station 195. Common on rocky shores at Scotsman Bay and found at Bass River.

Harmothoe imbricata (Linnaeus). At *Prince* stations 195 and 196; intertidal at Scotsman Bay and at Amethyst Cove, near Cape Blomidon.

Phyllodoce gracilis Verrill. Taken in large quantity at *Prince* station 195.

Eulalia gracilis Verrill. At *Prince* station 195.

Nereis pelagica Linnaeus. At *Prince* stations 190, 194 to 196, and at low water mark near Cape Blomidon.

Nereis virens Sars. Found under stones on mud flats at Scotsman Bay and Bass River.

Nephthys sp. Young specimens, thought to be *N. caeca* Gir., found on mud flats at Scotsman Bay.

Aricia ornata Verrill. A single specimen was found among rocks at extreme low tide at Scotsman Bay.

II. Polychaeta: Terebelliformia

Amphitrite sp. One specimen, probably *A. attenuata* Moore, was taken at low tide on rocky bottom at Scotsman Bay.

III. Polychaeta:Scoleciformia

Clymenella sp. Found at *Prince* stations 191 and 193.
Brada sp. Found at *Prince* stations 191 and 193.
Trophonia sp. Collected at *Prince* stations 191 and 193.
Sternaspis fossor Stimpson. Taken at *Prince* station 191.

ARTHROPODA:CRUSTACEA

I. Cirripedia

Balanus balanoides L. (Rock barnacle). Collected at M2, M8, M10, M12, M13, M17, M19, and observed on shore rocks at S16, M4, M15, and C15. Very common intertidally throughout the region but not found at the head of Cobequid Bay (M11, Maitland). On rocks, stones, shells, etc. Exuviae of this species were commonly taken in plankton tows.

B. improvisus Darwin (Little ivory barnacle). Collected in abundance at M2, M8, M10, M13, M15, M17, and observed at M12. A Minas Basin endemic, near and below LW level, but not taken in the Channel. On rocks, shells.

B. crenatus Brug. (Sharp-rimmed barnacle). Collected at M12, M17, C15, and M10, in 1950. Moderately common at and below LW level throughout the region, especially in Minas Channel and Bay of Fundy, on shells, etc.

B. balanus L. and *B. hameri* Ascan. Base rings of these deeper water species noted on the beach shells of *Placopecten, Volsella modiolus,* etc., from the Channel and Bay.

II. Mysidacea

Mysis stenolepis Smith. Obtained at Scotsman Bay and *Prince* 193, 195, and 196. Collected sparingly at M8 and C15 and M15 in 1950. A warmer water species usually associated with eel grass and lagoons.

Neomysis americana Smith. Rather more common, especially in the Basin, on sandy mud flats at M11, M12, M13, and M17, in 1950. Also taken in tows at *Prince* 190, 191, 193, 195, and 196. Partial to very low salinities (10‰).

III. Cumacea

(Identifications of 1921 material by Dr. W. T. Calman, British Museum of Natural History.)

Oxyurostylis smithi Calman. Probably the most common Canadian-Atlantic intertidal species; taken at M2, M17, M19, and C15, in fine sand, at LW level.

Eudorella truncatula (Bate) (=*E. pusilla*). Obtained in a towing net at *Prince* stations 191, 193, 195, 196. Females made up more than 90 per cent of mature specimens.

Diastylis sculptus Sars. One ovigerous female taken in fine sand, LW level, at C15. Very abundant at *Prince* 191, 193, 194, and 196, of which less than one per cent were males.

Lamprops quadriplicata Smith. Six adult and 4 imm. specimens taken at C15 and 5 females at *Prince* 195.

Leucon sp. One male specimen in surface tows at *Prince* 190.

IV. Isopoda

Ptilanthura tenuis Harger. One female, in fine sand, at C15. Appears to be a second Canadian record, the first being at Grand Manan (after Wallace, 1919).

Cirolana polita Harger. One immature specimen taken at C15. A cold-temperate species.

Jaera marina (Fabr.). A very common, widely distributed, intertidal species; taken at S14, S15, S17, M2, M8, M10, M12, M13, M15, M17, M19, C17, and C15; observed at S16 and at Amethyst Cove near Blomidon in 1921. There are several ecological subspecies known, of which two or more occur in Eastern Canada and are probably represented here. Males necessary for determination.

Idothea baltica (Pallas). A very common species, especially on fucoids, debris; common at Bass R. and Scotsman Bay and also taken at S14, M8, M15, M17, M19, C17.

I. phosphorea Harger. Less abundant and tending to warmer water localities than the above. Common at Bass River and Scotsman Bay, and at *Prince* 191 and 196. Immatures taken at M10, M15, C15, and adults at M13, M15 in 1950.

Chiridotea caeca (Say). Collected at mid-tide levels on sand beaches, especially in f.w. seeps, at M2 and M11, and M8, in 1950. A species of relatively ice-free shorelines.

C. tuftsi (Stimps). Far more abundant in the region than above. Taken at *Prince* 196 and at M2, M11, M12, M17, M19, and C15, in the lower tidal zone, on sand.

Edotea montosa (Stimps). Taken at M2, M19, and C15, burrowing in sand, and quite numerous at *Prince* 191, 193, 195, and 196.

V. Amphipoda

Tmetonyx nobilis (Stimps). A common sand-burrowing species of shore-line and shallows. Taken at M11, M15, M19, and C15; mainly immature specimens.

Orchomenella minuta (Kr.). Several specimens from shad stomachs.

Hippomedon serratus Holmes. Two immature specimens taken at C15.

Haustorius arenarius Slabber. Three specimens at C15, burrowing in fine sand. Taken only twice previously in Canada (Bousfield, 1956).

Bathyporeia quoddyensis Shoemaker. Scarce intertidally at M11 and M15. Known only from the Gulf of Maine. Taken at Bass R. in 1921 (Shoemaker, 1949).

Ampelisca spinipes Boeck. Rather common in sand and sandy mud at low water level throughout the Basin. Taken at M2, M8, M15, M19, and C15.

Phoxocephalus holbolli (Kr.). A circumpolar eurythermal species, taken in sizeable numbers at M19 and C15.

Sympleustes glaber (Boeck). One immature specimen taken at C15, LW level.

Stenopleustes gracilis Holmes. One ovig. female taken at M8 at LW. Common in the Bay of Fundy (Shoemaker, 1948).

Calliopius laeviusculus Kr. A very common coastal and littoral semi-pelagic species taken in numbers at M2, M8, M10, M11, M17, M19, C17, and C15.

Pontogeneia inermis Kr. A cold-water, free-swimming species of the lower intertidal region. Taken in the Channel at M19, C17, and C17.

Dexamine thea Boeck. Two specimens taken at C15. Likewise a cold-temperate species.

Gammarellus angulosus (Rathke). A species indicating uniformly cold-temperate and relatively clear waters of surf-exposed rocky coasts. Reaches the Channel only at S14, among algae at LW level.

Gammarus oceanicus Segerstrale. The most common intertidal amphipod; widely eurythermal and euryhaline; a summer breeder in Minas Basin. Taken at S14, S15, S16, M2, M8, M10, M11, M12, M13, M15, M17, M19, C17, and C15. Formerly known in America as *G. locusta* L. and *G. ornatus* M.-E.

G. setosus Dement. Two specimens taken in Scotsman Bay by A.H.L. in 1921, on shore. An arctic-boreal species not abundant in the Gulf of Maine.

G. lawrencianus Bousfield. A very common estuarine and intertidal species. Taken abundantly throughout the region; S14, S15, M2, M8, M11, M17, M19, and C17. Formerly known as *G. annulatus* in the Canadian Atlantic region.

G. tigrinus Sexton. A species of low-brackish tidal pools and headwaters of estuaries. Taken at S15, M8, M17 and observed at M6 and M7. Dominant in the Shubenacadie and Avon estuaries. (Formerly identified as *G. fasciatus* Say.)

G. mucronatus Say. A species of warm-water estuaries and salt-marsh pools. Restricted to and rather uncommon in Minas Basin; taken in the estuary at Five Islands (M17).

Rivulogammarus duebeni (Lillj.). Common in rock pools near HW level of wave-exposed coasts. Taken at S14, S15, and S17, but not inside the Basin.

Marinogammarus obtusatus Dahl. Taken at S14, S15, and M15, under stones and pebbles about mid-tide level, especially where free water trickles down the beach.

M. finmarchicus Dahl. Taken in tidal pools and under stones mainly near the top of the *Fucus* and barnacle zone, in the Bay and Channel at S14, S15, S17, M19 and C17, but not in the Basin proper.

M. stoerensis Reid. Taken near LW level at the mouth of streams tumbling down stony beaches, at S14 and C17, but not inside the Basin.

Melita dentata (Kr.). Taken in the Channel at C17 and C15, LW level.

Hyale nilssoni (Rathke). Under fucoids on rocks, etc., mid-tide level, at S14, S17, M8, M10, M13, M15, C15, and observed at C14.

Orchestia platensis (Kr.). The common beach flea of the entire region. Collected under wave-cast debris at the HW line at S15, M12, M15, and noted nearly everywhere else.

O. grillus Bosc. The salt-marsh hopper, rather common in the Basin, and collected at M2 and M10, among roots of salt marsh grasses at HW level.

O. gammarella (Pallas). Collected at the base of a rocky cliff at M15. A species of relatively ice-free coastlines and mild winters but rather unexpected so far up the Bay of Fundy.

Talorchestia longicornis (Say). The common sandhopper, taken on small sand beaches at M2, M4, M9, M10, all inside the Basin, but not seen along the north shore. Not previously recorded within the Bay of Fundy (Bousfield, 1958a).

Photis reinhardi (Kr.). Specimens noted in shad stomachs at Scotsman Bay (Shoemaker det.).

Amphithoe rubricata (Mont.). Taken sparingly under fucoids at S14, M8, M15, and M19.

Ischyrocerus anguipes (Kr.). Taken at S17, M19, and C15. A cold-water species of the lower tidal zone, under rocks.

Jassa falcata Montagu. Taken at C15. Habitat similar to preceding species but preferring somewhat warmer waters.

Leptocheirus pinguis (Stimpson). Sparingly taken on sand bottoms at M2, C17, and C15.

Corophium bonelli (M.-E.). Five ovig. females taken at C15. A cold-water littoral species reaching only to the mouth of the Channel.

Corophium volutator (Pallas). A dominant intertidal species of mud and sandy-mud bottoms, taken at M2, M8, M10, M11, M15, M17, and C17, and observed throughout the Basin. First recorded here by Kindle (1916) and later by Shoemaker (1947).

Corophium tuberculatum Shoemaker. A male and ovig. female were collected at LW level at M10; apparently a first Canadian record of this southern species (c.f. Shoemaker, 1947).

Unciola irrorata Say. Not uncommon on sand bottoms throughout the region; taken at M8, M10, and C15.

Caprella septentrionalis L. Taken at M19 and C15. A cold-water species of the Bay of Fundy reaching to the Channel.

VI. Decapoda

Eualus pusiolus Kr. Three specimens collected at LW level at C15 and a single ovigerous female in a rock pool in Scotsman Bay.

Crago septemspinosus (Say). The common sand shrimp of the entire region. Obtained in large numbers at *Prince* 190, 191, 192, 193, 195, 196. Very abundant at M2, M8, M10, M12, M15, M17, M19 (also C15 and M13 in 1950), but uncommon in the Bay of Fundy owing to unsuitable rocky coastline.

Homarus americanus M.-E. Evidence of lobster fishing activities noted along the Channel at S14, S16, S17, C14, and C15, but not inside the Basin. Taken only occasionally in the weir at Scotsman Bay.

Pagurus acadianus Benedict. One specimen taken at M19, in an old shell of *Buccinum*. Obtained at medium depth stations in the Channel and Basin but not in the very deepest localities at *Prince* 190 and 194.

P. longicarpus Say. Abundant at Scotsman Bay and at *Prince* 195, 196. Also collected abundantly at M2, M8, M10, M12, M13, M15, and M17 and observed commonly in the lower part of the tidal zone throughout the Basin. Utilizes all small gastropod shells, especially those of *Urosalpinx, Thais, Littorina* and *Lunatia*. Another Basin endemic, more recently recorded in Canada from Pubnico Harbour, western Nova Scotia (Bousfield, 1958b).

Cancer irroratus Say. The rock crab occurs sparingly throughout the region except near the head of Cobequid Bay. Taken at M2, M10, M19 and C17, and M13 and M15 in 1950.

C. borealis Stimpson. The Jonah crab is common in the Bay of Fundy and reaches the Channel but not the Basin. A cold-water species, often taken in lobster pots.

Carcinides maenas (L.). The green crab occurs throughout the region although not taken in 1921, 1950, nor known here prior to about 1953. Collected at S14, S15, S17, M2, M10, M13, M15, M17, M19, C17, and C15.

Ovalipes ocellatus (Herbst). Collected at Bass River and Scotsman Bay in 1922 for the first Canadian record and subsequently included by Rathbun (1929). Female lady crabs were taken in weirs at Bass River (M13) and Diligent River (C17), and a single large cheliped was found on the beach at Kingsport (M2). A warm-water Basin endemic.

Libinia emarginata Leach. Taken in the weir at Bass River (M13) and noted on shore at M10. A "southern" species of muddy estuaries, first recorded from Minas Basin by Kindle (1917).

Hyas coarctatus Leach. One specimen, 12 mm in length, was taken in a rock pool at low-water mark in Scotsman Bay and two specimens about 3 cm in length were dredged at *Prince* 195.

ARTHROPODA:ARACHNIDA

I. Pycnogonida

(Identifications by Professor L. J. Cole, University of Wisconsin.)

Nymphon rubrum Hodge. Several specimens were obtained at *Prince* 193, 195, and 196.

Phoxichilidium femoratum (Rathke). Taken at *Prince* 191 and 195.

Pycnogonum littorale (Strom). Three specimens were dredged at *Prince* 190, and a single specimen was taken intertidally at Bass River.

MOLLUSCA

(Previous distribution according to LaRocque, 1953, nomenclature acc. to Abbott, 1954)

I. Gastropoda

Acmaea testudinalis (L). Tortoise-shell limpet. Common intertidally throughout the region. Collected on rocks at S14, M10, M12, M19, C18, and C17, and at M13 and M15 in 1950.

Puncturella noachina (L.). One beach specimen only, taken at M19.

Margarites cinereus Couth. One beach shell taken at M19.

M. helicinus (Fabr.). Five specimens taken at S14; larval and juvenile shells at *Prince* 191 and Bass River. A cold water species of the lower tidal zone, not populating the Basin.

Skenea planorbis (Fabr.). One specimen at M15.

Lacuna vincta (Montagu). Taken in small numbers at S14, M8, M15, M19, and C17. A cold-temperate species of outer rocky beaches. Larval specimens, probably of this species, were obtained in tows at Bass River and *Prince* 191.

Littorina littorea L. The common or edible periwinkle of the entire region. Small specimens were sparingly taken at Scotsman Bay, Bass River, and Tennycape in 1921; collected or observed at virtually every station visited in 1958.

L. saxatilis Olivi. The rough periwinkle is commonly observed in the upper tidal zone of the Bay of Fundy and Minas Channel and throughout the tidal zone in Cobequid Bay.

L. obtusata L. The smooth periwinkle was collected sparingly at S14, M2, and M19, and observed on fucoids at M8, M10, M15, and C15; a single specimen inhabited by *Pagurus longicarpus* was taken at Tennycape in 1921.

Cingula aculeus Gould. Three specimens at C15. Four juvenile shells taken in a tow at Bass River.

Hydrobia minuta (Totten). A common salt-marsh species, taken at M2 and M17.

Crucibulum striatum (Say). Four specimens taken at LW level at M19, some on *Astarte castanea*, and others at *Prince* 191, 194, and 195.

Crepidula fornicata L. The common slipper limpet, widely distributed throughout the Basin and Channel but not in the Bay of Fundy. Collected at M2, M8, M10, M13, M17, C18, C17, and observed as a beach shell at nearly every other station. Also taken at *Prince* 195.

C. plana Say. The flat slipper limpet is less widely distributed but more abundant in Cobequid Bay than the above. Taken at *Prince* 192, 195, and 196, and at M2, M8, M15, M19, and C18. Commonly lining the inner wall of *Lunatia heros*, in which hermit crabs were also living.

Velutina laevigata (L.). Single specimens were taken at *Prince* 194, 195.

Polinices immaculata Totten. This small moon snail was represented by single specimens at M2 and M19.

Lunatia heros (Say). The common moon shell occurs throughout the region especially on sand bottoms; collected at M2, M10, M13, M15, M19, C18, and observed as beach shells elsewhere.

L. triseriata (Say). The three-lined moon snail is likewise very common intertidally throughout the Basin and the shell is utilized frequently by the hermit crab. Taken at Bass River, Scotsman Bay, *Prince* 192, 195, 196, and at M2, M8, M10, M12, M13, M15, M19, and C17.

Urosalpinx cinerea (Say). The oyster drill is one of the most common and conspicuous shells of the lower intertidal zone of Minas Basin and Cobequid Bay where it preys largely on barnacles and small bivalves. Collected at M8, M10, M12, M13, M15, and a beach shell at C18. A warm-water Basin endemic.

Thais lapillus L. The common dogwinkle occurs intertidally throughout the region except at the head of Cobequid Bay.

T. l. imbricatus Lamarck. This fluted variety was taken only in Minas Basin, at M2, M8, M10, M13, and M17; particularly common at Bass River.

Mitrella lunata (Say). Rather common near low water level throughout the Basin. Collected at M2, M8, M10, M11, M12, M15, and M17.

Buccinum undatum L. A cold-temperate species penetrating into Minas Basin from the Bay of Fundy. Taken at all *Prince* stations except P193; also at M8, M13, M19, C18, C17, and noted as a beach shell at stations along Minas Channel.

Colus stimpsoni (Mörch). As above; taken at C18, noted at C14.

C. pygmaeus (Gould). A beach shell, collected at C18.

Neptunea decemcostata (Say). A common beach shell of the Channel; taken at Scotsman Bay and at C18, C17; noted at C12, C14.

Nassarius trivittatus (Say). The channelled basket is a common beach shell throughout the Basin and was taken in every collection. Larval specimens taken in a tow at *Prince* 191.

Nassarius obsoletus (Say). The mud snail is a common intertidal species of the entire Basin; collected at *Prince* 192, 195, at Bass River, and at M2, M8, M10, M11, M12; partial to warm brackish water.

Lora nobilis Möller. Collected sparingly at M2, M8, and M19, sometimes inhabited by *Pagurus longicarpus*.

Lora bicarinata (Couthouy). Taken at LW level at M2, M17, and C15, often inhabited by *Pagurus longicarpus.*

Retusa pertenuis (Mighels). One specimen taken at C15, in sand, at LW level.

Cylichna alba (Brown). One specimen at C15, as above. Taken at *Prince* 191 and 193.

Odostomia trifida (Totten). Four specimens, under stones, intertidal, at M10.

O. bisuturalis (Say). Single specimens intertidally at M10, M17.

Turbonilla interrupta (Totten). Common near LW line of tidal flats at M2, M7, and M8. These last three species are warm-water Basin endemics.

Cepea hortensis Müller. Collected along shore at M6, M19.

II. Amphineura

Ischnochiton ruber (L.). Single specimens under rocks at LW at M19, C15. A cold-water species penetrating into Minas Channel from the Bay.

Ischnochiton albus L. Three examples were dredged at *Prince* 190.

III. Pelecypoda

Nucula proxima Say. The Atlantic nut clam was collected at M17 and M19 in sand at LW. Larval shells, presumably of this species, were taken at *Prince* 191, 193, 195.

Arcidae. Three separated valves were taken on the beach at M19. Possibly a fossil species eroded from bottom strata.

Crenella glandula Totten. Taken in fine sand at LW level, at M19, C15.

Volsella modiolus L. A cold-water sub-tidal species extending into the Basin from the Bay. A common beach shell nearly everywhere; taken at all *Prince* stations and at M2, M13, M19, C18.

Mytilus edulis L. The blue mussel is remarkably scarce in Minas Basin; no mussel beds were seen anywhere in the region. Isolated specimens taken at S14, M2, M10, M19, and M15 in 1950, and noted on the beach at M8, M12, M13, and C12. A few were dredged at *Prince* 190.

Musculus niger Gray. Separated valves taken along shore at M8, M19, and a single juvenile at Cape Blomidon.

M. laevigatus (Gray). Common at *Prince* 191, 194, 195, 196.

M. discors L. Collected at M8; isolated valves on beach at M17, M19.

M. corrugatus (Stimpson). Not uncommon at *Prince* 190, 191, 194, and 195.

Chlamys islandicus Müller. A single valve noted at LW at M15.

Placopecten magellanicus (Gmelin). Beach shells taken or observed at M19, C17, C13, C12, and dredged at *Prince* 191 and 192. Both scallop species are common in the Bay and are probably washed into the Channel and Basin by the strong deep currents.

Anomia aculeata Gmelin. Smooth form taken at the mouth of the Avon River, opposite Cheverie Pt., and at Scotsman Bay. One imm. specimen taken at C15 and separated valves (one of the smooth form) noted on the beach at M15, M19.

Astarte subequilatera Sowerby. One live specimen and dead valves taken at *Prince* 194.

A. undata Gould. A common beach shell of the Channel; taken at M19, C17, C15, and noted at C13. Dredged at *Prince* 191, 192, 195 and collected intertidally at Scotsman Bay.

A. castanea Say. The dominant beach shell at M19 (also taken alive at LW, on sandy mud) and noted at C17.

Venericardia borealis (Conrad). A moderately common beach shell along the Channel; taken off Kingsport and at M19, C17, C15, and noted at C13.

Arctica islandica (L.). Two specimens and two valves at *Prince* 192.

Cerastoderma pinnulatum (Conrad). Isolated valves taken in sand at M19 and C15.

Veneridae? Eight isolated valves of this strongly sculptured clam (one bored by a whelk) were collected on the beach at M19.

Pitar morrhuana (Linsley). From 1½ miles NE. of Kingsport to shore. A single valve was taken in the lower tidal zone at M2. A warm-temperate species and a Basin endemic.

Gemma gemma Totten. A species of warm-water salt-marsh pools and estuaries. Taken in sandy mud at M2 and noted at M12.

Petricola pholadiformis Lamarck. A characteristic and dominant rock-boring species of the lower tidal zone throughout the Basin. Abundant at Bass River and collected at M2, M8, M10, M11, M12, M15, M17, and M19.

Macoma balthica (L.). A common species of mud flats and estuaries, extending into nearly fresh water, throughout the region. Taken at M2, M8, M10, M11, M12, M13, M17, M19, C18, C17, and noted on the beach at M7, M15, and M18. Larval shells obtained in tows at *Prince* 191 and 193. Very common in the Shubenacadie and Avon estuaries.

Ensis directus (Conrad). The Atlantic razor clam was taken sparingly on sand flats at M2, M10, M13, M19, and C15, larval shells at *Prince* 193.

Spisula solidissima (Dillwyn). Separated valves taken at M2, M19, and noted on the beach at M8, M10, M13.

Hiatella arctica (L.). Two juvenile specimens were dredged at *Prince* 190. A single valve was noted on the beach at M10.

Mya arenaria L. The soft-shell clam is moderately common on mud and sandy mud flats throughout the region. Taken at every collecting locality in the Basin and noted as a beach shell nearly everywhere else.

Barnea truncata Say. The fallen angel wing was not uncommon at M10, M12, and noted at M13 (Cobequid Bay), boring in sandstone at the LW level with *Petricola*, but not nearly so abundant. A warm-water Basin endemic and first Canadian record.

Teredo navalis L. Water-logged wood and timbers, riddled presumably by this species, were noted in the drift line along the Channel and outer Basin; may have been carried in from the Bay of Fundy.

Lyonsia hyalina (Conrad). Collected rather commonly on sandy bottoms at LW level at M2, M8, M10, M17, M19, and observed at C17. More than thirty specimens taken at *Prince* 195. A southward-ranging species and Basin endemic.

Pandora gouldiana Dall. Not uncommon in the lower tidal zone of sandy mud beaches in the Basin. Taken at M8, M10, and M19.

Periploma leanum (Conrad). Not uncommon in sand at LW level at M19 and C15. A single valve taken on the beach at M17.

Elliptio complanatus (Dillw.). Three pairs of unseparated valves were found near LW level on the beach at M19. This freshwater species may have been ice-rafted from local estuaries.

IV. Cephalopoda

Loligo pealei LeSueur. Three specimens of the long-finned squid were taken in the weir at Bass R., June, 1950. An unusual Basin endemic, taken frequently in weirs at Scotsman Bay and Bass River in 1921, and forming the basis of Huntsman's (1924) record.

CHAETOGNATHA

Sagitta elegans Verrill. Found in plankton tows at *Prince* stations 191 and 195 and at Bass River.

ECHINODERMATA

(Identifications by Mr. A. H. Clark, United States National Museum)

Echinoderms are extremely rare on the foreshore of the region. Most of the species reported below were dredged.

Asterias vulgaris (Packard). One specimen from each of *Prince* stations 190 and 195 and on sand bottom at C15. Recorded 1½ miles northeast of Kingsport by Kindle.

Leptasterias littoralis (Stimpson). Small specimens were taken at all *Prince* stations 190 to 196, and a small one, 2 cm in diameter, was collected in the intertidal zone at Scotsman Bay.

Henricia sanguinolenta (O. F. Müller). At *Prince* stations 191, 192, 195, and 196. Reported 1½ miles northeast of Kingsport by Kindle.

Amphipholis squamata (Delle Chiaje). At *Prince* stations 191 and 195.

Strongylocentrotus drobachiensis (O. F. Müller). A very small specimen, 17 mm in diameter, at *Prince* station 191.

Echinarachnius parma (Lamarck). One small specimen, 17 mm in diameter, at *Prince* station 192.

BRYOZOA

Flustra foliacea (L). A very common beach shell, often washed ashore in heaps and frequently attached to *Volsella modiolus* and other bivalves. Occurs in dichotomously branching clumps of 3 to 8 inches in height.

BRACHIOPODA

(Identification by Dr. W. H. Dall, United States National Museum.)

Terebratulina septentrionalis (Couthouy). Abundant at *Prince* station 191 and found at 192 and 195.

TUNICATA

(Identifications by Dr. A. G. Huntsman, University of Toronto.)

Molgula citrina Ald. and Han. Two specimens at *Prince* stations 190 to 196.

Boltenia ovifera (Linnaeus). Sea potato. Taken at *Prince* stations 190 to 196.

Boltenia echinata hirsuta (Ag.) Two specimens at *Prince* station 191.

Amaroucium glabrum Verrill. Many large colonies at *Prince* station 195 and smaller ones at station 196.

Hydrographic and Marine Biological Features of the Area

On the basis of measurements and observations at shore localities in 1958, the study area may be separated hydrographically into three main regions, viz:

(1) Bay of Fundy, characterized by summer surface temperatures of less than 13°C., shore salinities of more than 31‰, relatively clear water (Secchi Disc depth approx. 15 to 30 feet), strong wave and surf action, but lowest tides (38 feet extreme rise of tide at HW springs).

(2) Minas Channel, characterized by geographically and bathymetrically uniform temperatures (13 to 15°C.) and shore salinities (30.5 to 30.9‰), rather turbid water (S. D. depth approx. 10 feet), reduced wave and surf action, and intermediate tides (45 feet extreme rise).

(3) Minas Basin, characterized by surface temperatures in excess of 15°C. and shore salinities less that 30.5‰, "lake-like" wave action and no surf, extremely turbid water (S. D. depth less than five feet), and highest tides (extreme rise of 53.3 feet at Burntcoat Head).

Minas Basin branches eastward and southward into two hydrographically and biologically distinct subregions, i.e., Cobequid Bay and the southern bight formed by the confluence of the Avon, Gaspereau, and Cornwallis estuaries, respectively. Because of influx of warm fresh water from the

Shubenacadie and Avon River systems and etxensive solar heating of exposed tidal flats, these two subregions are consistently warmer (17 to 20+°C.) and less salt (24 to 30‰) than the rest of the Basin and compare in these respects with other warm-brackish regions of the Bay of Fundy such as the St. John estuary, head of Chignecto Bay, and head of St. Mary Bay. The nearly land-locked situation of these two subregions and the dynamically stable circulation of the Basin are important factors in maintaining these conditions throughout the summer.

Accompanying these unusual hydrographic conditions is found a high proportion of endemic populations of animals whose normal geographic range is to the south of the Bay of Fundy or in the warm, brackish, southwest portion of the Gulf of St. Lawrence. Some of these endemic species appear to be separated from the nearest outside populations by distances beyond the normal range of larval transport; e.g. the medusa (*Nemopsis bachei*), the little ivory barnacle (*Balanus improvisus*), hermit crab (*Pagurus longicarpus*), lady crab (*Ovalipes ocellatus*), common spider crab (*Libinia emarginata*), flat slipper limpet (*Crepidula plana*), lunar dove shell (*Mitrella lunata*), pyramidellid snails (*Odostomia* spp., *Turbonilla interrupta*), rock-boring bivalves (*Petricola pholadiformis, Barnea truncata*), morrhuana Venus (*Pitar morrhuana*), and ribbed mussel (*Volsella demissus*). Huntsman (1952) listed the copepod species *Acartia tonsa* and *Pseudodiaptomus coronatus* among the holoplanktonic endemics, and Jermolajev (1958) added *Eurytemora herdmani* and a form of *Centropages hamatus.*

Certain species endemic to Minas Basin are also known from other "warm-water" but isolated localities in the Bay of Fundy region and elsewhere on the Canadian Atlantic Coast. These include the mysid shrimps *Neomysis americana* and *Mysis stenolepis*, a number of amphipods such as *Gammarus mucronatus, Orchestia grillus*, and *Talorchestia longicornis*, and tube-dwelling *Corophium volutator* and *C. tuberculatum,* the long-finned squid *Loligo pealei,* the slipper limpet *Crepidula fornicata,* the three-lined moon snail *Lunatia triseriata,* the oyster drill *Urosalpinx cinerea,* the mud snail *Nassarius obsoletus,* the bivalves *Gemma gemma, Lyonsia hyalina,* and *Pandora gouldiana,* and the sand flounder *Lophopsetta maculata.*

The maintenance of holo- and mero-planktonic populations in Minas Basin appears rather remarkable. Ketchum *et al.* (1953) point out that tidal exchange ratios of various parts of an estuary may be used to predict the distribution of free-floating or planktonic organisms in much the same manner as the distribution of fresh water in the estuary. As a result of circulation alone, the population of planktonic animals "P_m" remaining after being depleted from its initial size "P_o" by a series of tidal cycles and not receiving recruitments from upstream or downstream during the interval is given by

$$P_m = P_o\ (1-r)^m$$

in which "m" is the number of tidal cycles and "r" is the exchange ratio, defined as the proportion of water moving seaward during each tidal cycle which does not return on the following flood. Using this equation and on these assumptions, Bousfield (1955b) calculated that an exchange ratio of 0.15 in the Miramichi estuary would be sufficiently high to prevent the

maintenance of *Balanus improvisus* having a fecundity of 10,000 larvae per adult and a planktonic duration of 18 days or 36 tidal cycles, at prevailing sea temperatures of 20°C. Within this estuary, a river-induced non-tidal drift, seawards at the surface and landwards near the bottom, in conjunction with changing vertical distribution of successive larval stages provided the mechanism necessary for larval retention and maintenance of the population of barnacles and other organisms.

In Minas Basin, however, the exchange ratios in $4\frac{1}{2}$ tidal segments estimated by Ketchum *et al.* (1953) are theoretically much larger (0.39 to 0.94), and a residual two-layered drift circulation is virtually non-existent because of the relatively small fresh-water influx and intense tidal mixing (p. 3). Planktonic retention of many organisms within the Basin would thus appear unlikely under the conditions stated and assumptions made. How then do we account for the fact that the previously mentioned species do maintain sizeable populations well within Minas Basin?

The solution to this problem is indicated by the "Sargasso Sea" effect observed in Minas Channel (p. 2). The proper application of the simplified tidal exchange equation requires that the water flowing out of the Basin and mixing with outside waters on the ebb does not return on the flood. Owing to the trough-like configuration of the Bay of Fundy and Minas Channel there are no sustained cross-currents to carry the ebb water laterally away from the mouth of the channel. Moreover, the landward set of tidal currents along the south shore of the Bay of Fundy, under the Coriolis effect, accentuates the "bottling up" of Minas waters. Thus, virtually the same water, albeit thoroughly mixed vertically, returns on the flood: the tidal cycle creates a simple back-and-forth flow of the entire water column within the confines of the Channel and Basin. Also, the relatively small influx of fresh water results in a small net seaward flow from the Basin into the Bay. Thus larvae spawned in the headwaters remain largely within the Basin until completion of plantonic development.

Strong vertical mixing by the tides maintains a high degree of turbulence and water turbidity in Minas Basin, thereby seriously reducing the surface zone of photosynthetic activity and preventing extensive development of phytoplankton. Huntsman (1952), however, noted very large populations of endemic copepods available as food for fishes toward the head of Cobequid Bay and in the Shubenacadie estuary. He postulated that the amount of growth of the phytoplankton, on which the zooplankton feed, should increase in the headwaters of the Basin, since the shallower the water the less far they will be taken from the surface by the turbulence. Presumably then, food material for fishes and benthonic invertebrates in the Basin is derived very largely from endemic phyto- and zoo-plankton in the headwater estuaries and to a lesser extent from organisms and organic debris brought in from the Bay of Fundy by tidal mixing.

It is not surprising, therefore, to find among the most conspicuous and abundant bottom invertebrates many detritus or deposit feeders (e.g. cumaceans, mysid shrimps, burrowing amphipods (*Ampelisca, Bathyporeia, Corophium, Unciola*) and isopods (*Chiridotea, Edotea*) and bivalves (*Macoma balthica, Lyonsia hyalina*)); scavengers (e.g. *Crago, Pagurus, Cancer, Libinia*, and gammarid amphipods); ecto-parasites (pyramidellid snails);

and predators (e.g. *Urosalpinx, Thais, Lunatia, Loligo, Ovalipes, Carcinides,* and nereid worms). Many suspension or filter-feeding intertidal animals that might otherwise be expected in the Basin are relatively scarce (e.g. *Mytilis edulis, Volsella demissus, Pitar morrhuana, Teredo*) or lacking altogether (e.g. *Crassostrea virginica, Venus mercenaria, Mulinia lateralis*), although a few are abundant (e.g. *Mya arenaria, Petricola pholadiformis, Crepidula* spp., and *Balanus* spp.). The scarcity of intertidal echinoderms, despite the high salinity, is a noteworthy peculiarity of the region, which may be related to the scarcity of principal food organisms (e.g. *Mytilus*), lack of suitable cover, inability of the larvae to survive in the Basin, or other unknown factors. The absence of eel grass (*Zostera marina* L.) and its associated microflora and fauna (e.g. cerithiid snails (*Bittium*), the amphipods (*Corophium insidiosum, Caprella geometrica, Amphithoe longimana*), and decapods (*Palaemonetes vulgaris* and *Neopanopeus texana sayi* etc.)) may result from sub-minimal light conditions for photosynthesis at the appropriate tidal levels, caused by extreme water turbidity and the virtual absence of standing water at these levels when the tide is out. Brackish pools among the *Spartina* and other salt marsh grasses near the HW level are crowded with *Gammarus mucronatus*, a small corixid water bug, and the snail *Hydrobia minuta*, with *Orchestia grillus* at the edges, and *Gammarus tigrinus* and *Corophium volutator* where fresh water seeps in. However, the ribbed mussel *Volsella demissa* (recorded by Whiteaves, 1901) and the air-breathing marsh snail *Melampus lineatus* were not found during limited search.

Certain intertidal species are extremely abundant in the Basin and Channel, much more so than along the outer Fundy shores or elsewhere in Eastern Canada. The common rock barnacle, *Balanus balanoides*, settles on all rock surfaces so completely, that in June and July the rocks appear coated with a white powder, from about 10 feet below the extreme HW line to near LW level. A similar concentration was noted at Hall's Harbour by Stephenson *et al.* (1954). The horizontal range of rocky tidal flats (one-half to three-quarters of a mile) and high proportion of intertidal to subtidal bottom (p. 3) result in a very large population of intertidal barnacles and an enormous concentration of larval stages in the Basin during the spawning season. These evidently stay mainly within the Basin during their 30-day planktonic period, and then saturate every available surface at the time of attachment. The presence of these barnacles is no doubt largely responsible for the corresponding intensity of populations of *Urosalpinx, Thais,* and other predatory whelks.

Minas Channel, on the other hand, marks the seaward limit of the Basin endemics and the landward limit of shore-dwelling invertebrates characteristic of the relatively cold salt waters of the Bay of Fundy. Among these last may be mentioned the free-swimming amphipods *Gammarellus angulosus, Pontogeneia inermis, Gammarus setosus,* and *Marinogammarus stoerensis,* the rock-clinging *Ischyrocerus anguipes,* and *Caprella septentrionalis,* and burrowing *Corophium bonelli* and *Hippomedon serratus;* the chitons *Ischnochiton ruber* and *I. albus* and several gastropods including *Lacuna vincta, Margarites* spp., *Crucibulum striatum,* and *Cingula aculeus.*

The establishment in the lower tidal zone of many deep-water bivalves and gastropods (e.g. *Astarte* spp., *Venericardia, Periploma, Nucula, Crenella, Buccinum,* and *Lora* spp.) is probably attributable to the bathymetrically uniform temperatures and to the vertical transport of larval stages by strong tidal currents. These currents are likewise responsible for casting ashore the shells of these and other deep-water species (e.g. *Placopecten, Puncturella, Balanus balanus* and *B. hameri, Colus* and *Neptunea*) on sand and gravel beaches lining the Channel.

Finally, we might note that several species taken during the course of this work are new to the Canadian Atlantic coast (e.g. *Nemopsis bachei, Corophium tuberculatum, Thais lapillus imbricata,* and *Barnea truncata*) or recorded previously in Canada only here (e.g. *Loligo pealei, Corophium volutator, Pagurus longicarpus,* and *Libinia emarginata*).

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SOME RADIOGRAPHIC OBSERVATIONS ON THE GASTRO-INTESTINAL AND URINARY SYSTEMS OF ANESTHETIZED PACIFIC SALMON (ONCORHYNCHUS)[1]

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Abstract

Standard radiography with barium sulphate as the opaque medium was used to show the gastrointestinal and urinary systems of some intact, living *Oncorhynchus* species. The fish were anesthetized and the barium introduced into the systems by means of a syringe fitted with fine polyethylene tubing. Lateral and ventral views are presented in a series of photographs.

Introduction

There have been no published, detailed anatomic studies of *Oncorhynchus* species since the pioneer work of Green (1), despite years of intensive research on these fishes, and apparently no one has attempted to use radiography to study non-osseous structures in these fish. The advantages of studying functional rather than post-mortem anatomy are manifold. Radiography offers one of the most convenient methods of elucidating functional anatomy.

This paper, the outcome of a short investigation designed to clarify the shape and position of structures involved in proposed physiological studies, was considered of interest because of the paucity of appropriate anatomic data concerning Pacific salmon. Radiographic techniques will doubtless be fruitful in revealing many other anatomic features and functions in both intact fish and in excised tissues. The work of Mott (2) on the circulatory system of the eel elegantly illustrates this potential. It is hoped that this report will serve to demonstrate a useful technique and to stimulate further investigation of the anatomy of *Oncorhynchus* species.

Materials and Methods

In general, routine radiographic methods were employed but the $BaSO_4$ suspension used as the opaque medium was made very finely divided by precipitation from $BaCl_2$ with H_2SO_4. The precipitate was then washed repeatedly with water and stored in water. A flexible, opaque probe made by filling polyethylene tubing (0.011 in. I.D. × 0.024 in. O.D.) with barium sulphate suspension and sealing off both ends in a flame was used for preliminary radiographic examinations.

Exposures were made at approximately 55 kv and 40 milliampere-seconds using medical No-Screen film and a diagnostic X-ray unit.

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Contribution from the Fisheries Research Board of Canada, Biological Station, Nanaimo, B.C.
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Gastrointestinal Radiography

Two-year-old sockeye (*O. nerka*) and coho (*O. kisutch*) salmon which had not been fed for 48 hours prior to treatment were anesthetized in a 1:20,000 solution of tricaine methanesulphonate (MS-222, Sandoz Pharmaceutical Co.), and barium sulphate was forced into the stomach by means of a 10-ml syringe fitted with an adapter and polyethylene tubing (Clay-Adams Inc.) until excess barium appeared in the mouth. The fish were then returned to water for various periods and anesthetized again before X-ray examination.

Urinary Radiography

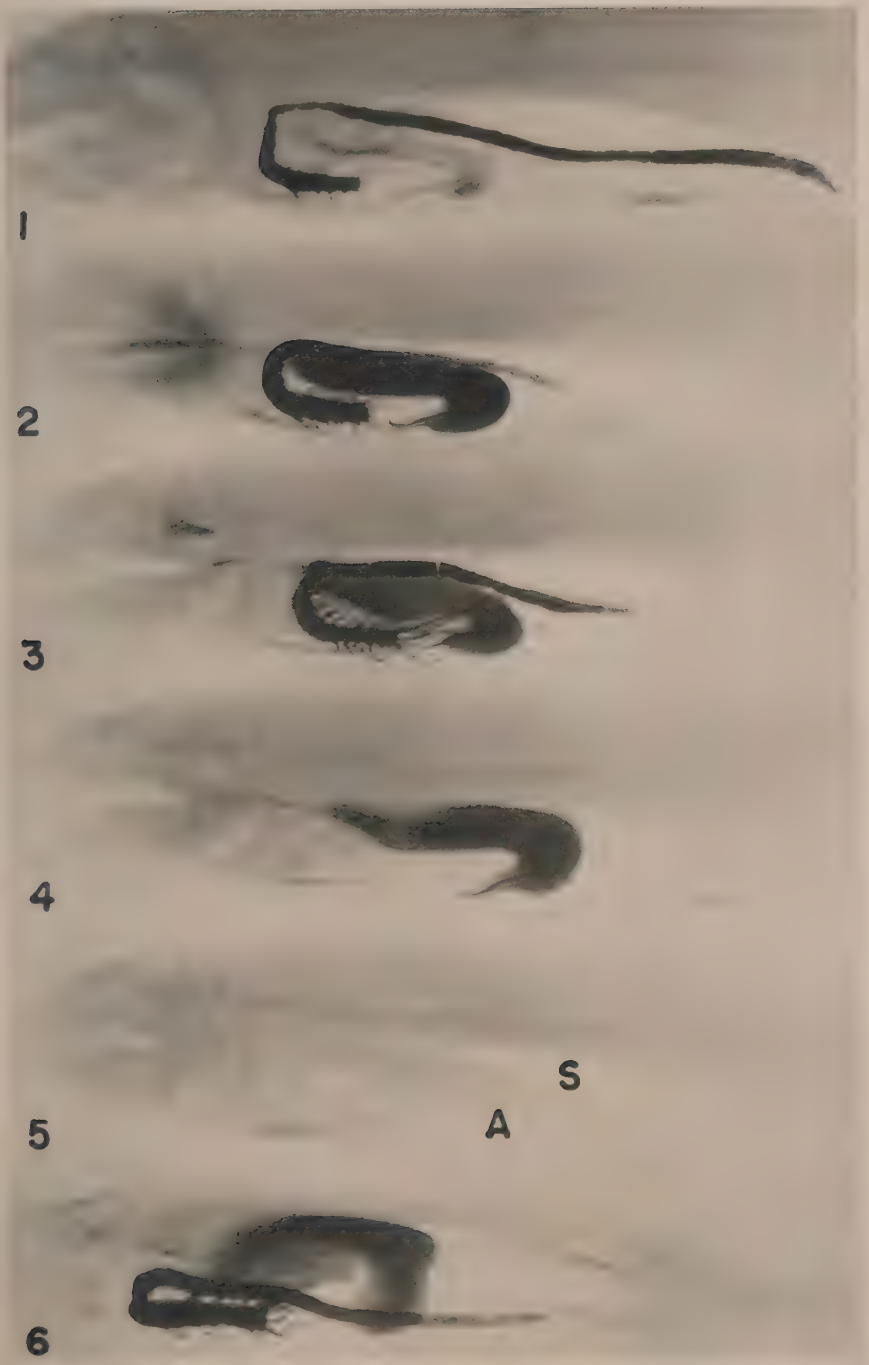
Barium sulphate was introduced into the urinary tract by carefully working fine polyethylene tubing attached to a syringe up the posterior opening of the urogenital papilla while the anesthetized fish lay on its right side. If the genital system were entered, the tubing could be felt to curve sharply toward the head and follow the length of the body but if the urinary system were entered, the tubing curved only slightly toward the head and was directed dorsally. Once in the urinary system the tubing was worked gently along the duct until it would go no further, then the barium sulphate was injected slowly while the tubing was gradually withdrawn. Care should be taken to round the edges of the tubing, otherwise they may tend to cut fine structures.

Results and Discussion

Gastrointestinal System

The results of the forced ingestion of barium sulphate by several fish are shown in Figs. 1–6 where the position of the barium is revealed by the very dark areas not present in Fig. 5, the untreated fish. In Fig. 1 it can be seen that some of the barium has passed completely through the gastrointestinal system and some has been trapped in the folds of the stomach musculature. The irregularities of the intestinal mucosa are also visible. Figures 1 and 2 show most clearly the constriction of the lower outlet of the stomach which might be termed the pyloric sphincter. In Fig. 2 some barium is seen still adhering to the mucosa of the lower intestine. The pyloric caeca, which help to increase the absorptive area of the otherwise short intestine, are shown particularly well in Fig. 3. It would be interesting to know how the food is passed through and out of these blind tubes. The shape of the stomach and the position of the esophagus are best illustrated in Fig. 4. Figure 6 helps to complete a three-dimensional "picture" of the gastrointestinal system but it is well to bear in mind that the anatomy of the system is modified

FIGS. 1–6. Radiographs of several anesthetized sockeye salmon (*O. nerka*) following the forced feeding of barium sulphate. The position of the barium in the gastrointestinal system is shown by the intensely dark areas.
FIG. 1. About 5½ hours after feeding.
FIG. 2. About 5 hours after feeding.
FIG. 3. About 3 hours after feeding.
FIG. 4. About ½ hour after feeding.
FIG. 5. Untreated fish.
FIG. 6. Ventral view at about 5 hours after feeding.
ABBREVIATIONS: S, swim bladder; A, probably ingested air.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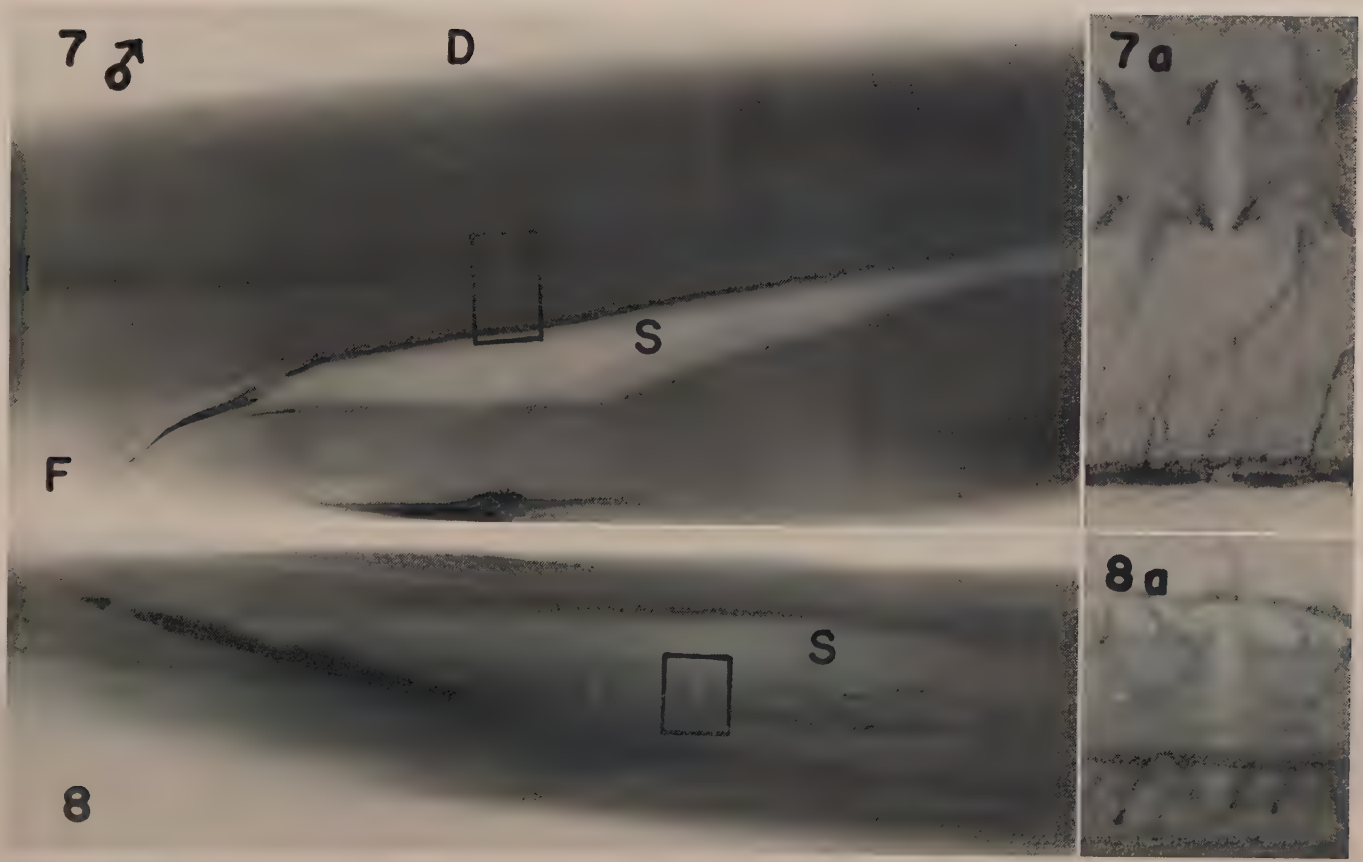
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8a

according to the position of the body, as is the case in humans. However, it can be seen from Figs. 1, 2, 3, and 6 that the stomach tends to lie sinistrally, then loops anterodextrally and ventrally so that the loop of the intestine having the pyloric caeca lies dextrally. The caecal portion of the intestine then loops posterodorsally and the straight intestine continues dextrally to the anus.

Using the above technique in conjunction with fluoroscopy, it also may be possible to study peristalsis and residence times of food in parts of the gastrointestinal system while the fish are being held in retaining apparatus such as that described by Mott (2). In this regard, the behavior of mature, non-feeding salmon would be of interest.

Urinary System

Parker (3) showed that in the brown trout (*S. fario*) the urinary and genital systems are entirely separate, thus correcting the common misconception that the two join posteriorly to form a single duct. According to our radiographic observations (Fig. 7) and those following latex injection of the two systems, this also appears to be true in both male and female coho salmon and is probably so in all *Oncorhynchus* species. In Fig. 7 the probe is shown in a sperm duct and the free barium in the urinary ducts. The kidney (or fused kidneys?) lies just ventral to the spine and runs the length of the body cavity and beyond to the pharyngeal region. Figures 7 and 8 show two mesonephric ducts with collecting tubules (Figs. 7*a* and 8*a*), which unite posteriorly and drain into a widening of the duct. The duct then continues posteroventrally and along the right side of the body cavity to terminate in the urogenital papilla posterior to the anus. The widening of the duct might be termed a bladder but its urine-storing function, with sphincter control, must first be established. The storage of urine by fishes might at first appear useless but it is possible that they need at times to withhold urine for resorption, or even in order not to attract predators.

It is remarkable that the barium penetrated the fine structures of this system but it is also possible that the opaque medium did not enter some significant ducts. "Miokon" and "Diodrast" which are used for urinary radiography in humans have so far been unsatisfactory for this purpose in salmon largely because there was much leakage from the site of injection, viz., the heart.

FIGS. 7 and 8. Radiographs of troll-caught coho (*O. kisutch*) 'grilse' following the injection of barium sulphate into the urinary system.

FIG. 7. The fine line of the barium-filled polyethylene tubing can be seen as the tubing lies in the lower end of the genital system, just ventral to the posterior end of the swim bladder. The free barium in the urinary ducts shows as dark areas and lines beginning posterodorsally to the barium probe and extending along the ventral surface of the kidney but apparently not into the head kidney region.

FIG. 7*a*. Photographic enlargement of area designated in Fig. 7.

FIG. 8. The same fish as in Fig. 7 shown dorsally. Here the two mesonephric ducts with their branches appear as fine lines underneath and to the side of the spine. The fish is lying slightly to the right of vertical.

FIG. 8*a*. Photographic enlargement of area outlined in Fig. 8.

ABBREVIATIONS: S, swim bladder; F, anal fin; D, dorsal fin.

Summary and Conclusions

The gastrointestinal and urinary systems of intact, living sockeye (*O. nerka*) and coho (*O. kisutch*) salmon respectively have been examined by radiographic techniques. The position of the gastrointestinal system, musculature of the stomach, and probable existence of a pyloric sphincter are shown. Mesonephric ducts to the kidney are also shown, as well as a widening in the common duct, perhaps describable as a bladder if this function can be established. It is probable that the urinary and genital systems of *Oncorhynchus* species are separate; however, all species have yet to be examined.

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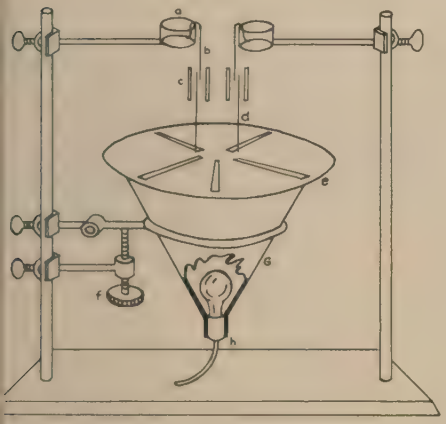
Time saving applications in horizontal paper chromatography of amino acids

e method published previously by the authors[1] separates the mixture of amino ids with the first solvent system into several groups, transfers these onto another per by capillary elution and effects the separation of the groups into individual mino acids with other solvent systems.

This method has been improved. A solvent system[2] was found which eliminates te need for rechromatography and a simple apparatus which dries the spots during te process of elution permits one worker to control six such procedures simultaneously (Fig. 1).

The dimensions of the paper for the separation into groups are given in Fig. 2.



g. 1. Simultaneous elution and drying of "group" K.C.T. paper. (a) Solvent vessel; (b) leader ip; (c) microscope slides; (d) cut out band om first chromatogram; (e) K.C.T. paper; (f) justment; (g) light metal cone, base and height 24 cm; (h) 40 W bulb.

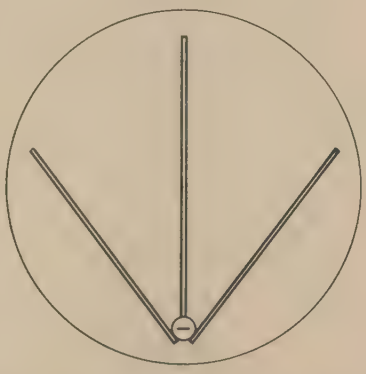


Fig. 2. Circular paper with two segments, one for ninhydrin development the other for cutting out and eluting groups of amino acids. Diameter 33.3 cm, of small circle 2.2 cm; slit in same 2 cm, paper tongue 2 × 5 cm; angles 37° each.

he chromatogram is run with the solvent system *n*-butanol–methyl ethyl ketone–ethylamine–water (20:20:2:10, v/v) which travels about 230 mm within 18 h at °. This is very convenient, permitting to start the run in the late afternoon and to ke it off next morning.

It was found advisable to increase the size of the box slightly to 37.6 × 37.6 × 7.5 n and to saturate the atmosphere therein by an additional 40 ml solvent, divided in ve regularly spaced, flat dishes. The paper is supported by glass needles of 4 cm ngth.

Diethylamine should be removed quantitatively in order to avoid complications uring the separation of the groups. This is achieved by drying the paper in a ventited oven at 70° for 30 min and by washing it three times with chloroform.

The distribution of the amino acids and the separation of the various grou with lutidine–water (3:2, v/v) and *tert.*-butanol–formic acid–water (75:0.8:24 v/v) are shown in Table I.

TABLE I

GROUP-SEPARATION AND SUB-SEPARATION OF AMINO ACIDS BY HORIZONTAL PAPER CHROMATOGRAP

Group-separation			*Sub-separation*	
BuOH–Me Et ketone–H_2O–diethylamine (20:20:10:2, v/v)			*Lut–H_2O (3:2, v/v)*	*tert.-BuOH–HCOOH– (75:0.8:24.2, v/v)*
Group	*amino acids*	R_F	R_F	R_F
I	Cystine	10–16		17–19
	Arginine			23–34
	Asp. acid			40–44
	Glut. acid			50–54
II	Cysteic acid	18–21	44–50	
	Lysine*		13–20	
III	Glycine*	23–25	37–41	
	Methiosulfone		45–49	
IV	Alanine	30–37		46–50
	Histidine			18–21
	Serine			38–41
V	Tyrosine*	42–44		
VI	Valine	47–50	59–64	
	Methionine		48–53	
VII	Threonine	57–69		45–49
	Phenylalanine			67–70
	Leucine and isoleucine			75–80

* Determined immediately after the first run, provided no H_2O_2 has been used.

Lysine, glycine and tyrosine appear as single bands and can be determin immediately after the first run. If, however, the sample spot has been treated with drop of H_2O_2, cysteic acid will appear with lysine (group II) and methiosulfone wi glycine (group III). Each pair is easily separated by lutidine–water (3:2, v/v) as sho in Table I.

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OCEANOGRAPHY IN CANADA 1957-60

by

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INTRODUCTION:

The Canadian Committee on Oceanography, as of December, 1959, is the re-organized Canadian Joint Committee on Oceanography which came into existence on April 1, 1946. Since 1946 oceanography in Canada has been co-ordinated under such a Committee which has been representative of all government agencies actively interested in the problems of the sea.

In the recent re-organization, the interests of the Committee have been broadened and its functions are now to:

(a) Co-ordinate the oceanographic activities of all Canadian Government agencies.

(b) Act, as designated by the National Research Council (NRC), as the National Committee for the Scientific Committee on Oceanographic Research (SCOR).

(c) Act, as designated by the National Research Council, as the Section on Oceanography of the NRC Associate Committee on Geodesy and Geophysics.

The Committee is comprised of representatives of the following Government Agencies:

The Royal Canadian Navy
The Fisheries Research Board
The Department of Mines and Technical Surveys
The Defence Research Board
The National Research Council
The Meteorological Branch, Department of Transport
The Marine Services, Department of Transport.

In addition there are representatives of academic interests in oceanography as nominated by the National Research Council.

Each of the co-operating organizations is either actively engaged in oceanographic investigations or contributes in ships, laboratory facilities, personnel, or funds towards the overall Canadian program. The total effort is co-ordinated through the Committee.

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There is an Institute of Oceanography at the University of British Columbia, Vancouver, B.C., under the directorship of Dr. G. L. Pickard, and an Institute of Oceanography at Dalhousie University, Halifax, N.S., under the directorship of Dr. F. R. Hayes. A Geophysical Research Group under the chairmanship of Dr. G. B. Langford at the University of Toronto, Toronto, Ont., is applying oceanographic methods to studies of our great inland seas. All three organizations are supported in part by funds from the National Research Council. The Departments of Zoology and Physics at McGill University, Montreal, P.Q., have research interests in marine biology and the physics of sea ice respectively. Problems in marine biology are also furthered by the Fisheries Institute of the University of British Columbia at Vancouver, B.C., the Regional Laboratory of the National Research Council at Halifax, N.S., and the School of Fisheries at Ste. Anne de la Pocatière, P.Q. The Department of Geology at Carleton University in Ottawa, Ont., and at the University of Montreal in Montreal, P.Q., are developing interests in phases of submarine geology. The Pacific Naval Laboratory at Esquimalt, B.C., and the Naval Research Establishment at Dartmouth, N.S., under the Defence Research Board conduct oceanographic research and surveys in support of military requirements.

The Fisheries Research Board, which serves as the scientific arm of the Federal Department of Fisheries, has been actively engaged in all phases of oceanography since 1910, with considerable impetus being given to the subject following the Canadian Fisheries Expedition of 1914–15 under Dr. Johan Hjort. Present activities are furthered by the Atlantic Oceanographic Group located at Halifax, N.S., the Pacific Oceanographic Group at Nanaimo, B.C., the Arctic Unit at Montreal, P.Q., and the Biological Stations at St. John's, Nfld., St. Andrews, N.B., and Nanaimo, B.C.

The Canadian Hydrographic Service of the Department of Mines and Technical Surveys has, from the time of Dr. Bell Dawson, pioneered in the study of tides and currents in areas of Canadian interest. Within the past two years, the Department of Mines and Technical Surveys has entered the field of oceanography on a more substantial scale. To meet requirements for greater attention to Arctic areas of national concern, a Polar Continental Shelf Project has been set up under the expanding plans. The Department also has under way the development of the Bedford Institute of Oceanography in Dartmouth, N.S., which is to be completed in five years. This Institute will house a staff of 300 oceanographers, hydrographers, submarine geologists, and other scientific personnel plus a supporting staff. The operating fleet will consist of ten oceanographic vessels. A multi-million-dollar ship building program is under way to provide the fleet of oceanographic vessels. The first of these, the $7,000,000 C.G.S.*Hudson,* is expected to be commissioned in 1961. Specifically, the new Institute will turn an oceanographic research spotlight on Canada's Atlantic and sub-Arctic waters. It will also permit expansion of hydrographic programs. The regional offices of the Geological Survey will be located in the Institute. It will also house the staff of the Polar Continental Shelf Project as well as the Atlantic Oceanographic Group of the Fisheries Research Board. The whole project will mean the building up, near

Dartmouth, of a strong center of marine sciences. It includes close co-operation with the Fisheries Research Board, with the Institute of Oceanography at Dalhousie University, with the Atlantic Regional Laboratory of the National Research Council, and with the Naval Research Establishment of the Defence Research Board. The overall development of the Bedford Institute will be in charge of Dr. W. M. Cameron.

The Province of Quebec maintains a Marine Biological Station at Grand River, P.Q., which gives attention to marine biological problems in the Gulf of St. Lawrence. Particular attention has been directed towards the general oceanography of the Bay of Chaleur.

At this stage of developments in oceanography in Canada, a most serious shortage of professional oceanographers exists, particularly in the physical and chemical disciplines. It is expected that as these developments in oceanography gradually come to fruition over the next five years, the required basic staffs can be built up with professional oceanographers who might be attracted from other areas, with repatriated Canadian professionals, and with the increasing number of graduates from our two Institutes of Oceanography.

The Canadian Oceanographic Problem

Canada has an enormous coast line, being bounded north, east, and west by three oceans, which are sources of food, means of transportation, factors in climate, and aids to defence.

As a source of food, the oceans present us with increasing economic and conservation problems as the world needs for protein brings greater pressure on fish stocks that at one time were considered inexhaustible. The major fish stocks off both the east and west coasts of Canada are being exploited as never before. The many international fisheries commissions now furthering research in these areas of Canadian concern indicate the importance that is attached to these ocean resources. While there are yet untapped resources of the sea, the economics of some of our present fisheries are of some concern to Canada at the present time. Some fisheries are not exploited to the full, and the efficiency of catching leaves much to be desired in others.

As a means of transportation, the oceans present a host of problems over a varied and extended coast line. Here the tides, currents, water characteristics and bottom configurations combine with wind, waves, swell, ice and icebergs to present a maze of challenging requirements for safe navigation, for the construction of wharves, harbours, causeways and bridges, and for defence. The recent developments of the large ore bodies in eastern Quebec, the industrialization of areas around the northern part of the Gulf of St. Lawrence, the operation of the St. Lawrence Deep Sea Waterway, and the requirements for sea-borne traffic in the Arctic have crystallized national requirements for a thorough study of ice in navigable waters. These studies which have recently expanded have already opened up further problems in the physics of ice, meteorology, and even in the design of ships for navigation through or under the ice.

As a factor in governing climate, the oceans are an important phase in the cycle of evaporation, cloud formation and precipitation. With

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oceans on three coasts, the interactions between sea and atmosphere are fundamental processes, and eventually must enter as parameters in long-range weather forecasts. While the waters on our northern coastline have ice cover for the greater part of the year, meteorologists have observed for example the very marked effect of the waters of Hudson Bay as conditions change from ice cover to open water and vice versa. However the problem is more than local, and involves the interactions of sea and atmosphere on a world-wide basis.

The oceans can still be considered an effective item of continental defence. They can also serve as an effective cover for under-sea craft which can remain submerged for long periods and serve as launching platforms for long-range missiles. Until recent years, the North American Continent, relatively untouched by modern wars, was looked upon as a bastion, well removed and protected from warring nations by the oceans on three coasts. In particular, the Arctic coast could be forgotten because of the difficulties of operation. Navigation under the Arctic ice by atomic-powered submarines has been shown to be feasible. This in itself has opened up a host of problems relating to defence. Methods of detecting the modern submarine are much more involved than heretofore, and a very technical knowledge of the ocean itself is required for efficient application of modern defence methods.

On the Atlantic coast the main water body of particular Canadian concern is contained within the area of confluence of two great ocean systems, the Gulf Stream and the Labrador Current. Thus the characteristics of the waters on our eastern sea coast are determined in the main by the mixing and layering of contributions from these two water masses, and modified in coastal areas by the contribution from land drainage systems. Of the land drainage systems, that of the St. Lawrence predominates, representing as it does the drainage from almost half a continent. The water areas within two hundred miles or more of our eastern coast support an enormous fishery, which has been known and exploited for centuries. The great contrasts in the waters of the Gulf Stream and the Labrador Current, the climatic variations in the mid-latitudes, the seasonal changes in the wind systems, the variety in the configuration of the sea floor and the coastline, the contrasting types of tide, the ice coverage, and drift of icebergs, establish a pattern of environments with a wide variety of parameters. To the oceanographer, whether interested in problems of food production, of transportation, of meteorology, or of defence, the area offers a particular challenge in many of its fundamental aspects.

In the northern latitudes of the northern hemisphere, the land masses offer considerable barriers to the exchange of waters as between the polar seas and the Atlantic and Pacific Oceans. The annual water balance of the Arctic Ocean has been calculated in which the inflow and outflow of waters through the Faeroe and Shetland Islands and the Danish and Bering Straits, and the continental run-off, have been taken into account. It has been indicated that there is a surplus of 31,400 cubic kilometres of inflowing waters which must be carried away annually from the Arctic Ocean through the numerous straits of the Canadian Archipelago. Hence on the Atlantic seaboard and in the eastern Canadian

Arctic, this outflow through Foxe Basin, Hudson Strait, Lancaster, Jones and Smith Sounds, and Kennedy Channel accelerate the Baffin Land and Labrador Currents which transport these Arctic waters with their ice and iceberg to the mid-latitudes of the Canadian Atlantic. In the western Canadian Arctic, west of Barrow Strait, the deeper waters are characteristic of those of the Polar Basin. The Canadian oceanographic problem in these far northern areas is concerned with the food resources of the sea for an Eskimo population, with navigation through ice-invested waters relating to supply missions, with development of natural resources, and with phases of continental defence.

The rugged Pacific Coast of Canada is indented with many deep inlets which present water bodies of various magnitudes with fjord-like characteristics. The oceanography of the immediate coastal seas is dominated by the outflow from the larger river systems and by the turbulent tidal currents in many straits and passages. The basic features of the ocean circulation in the North Pacific is the North Pacific Current, a part of the Kuroshio System. This North Pacific Current or west wind drift is the general eastward flow of comparatively warm water to the east of Longitude 160°E. To the north of the North Pacific Current and centred at about Latitude 50°N is found an entirely different sub-Arctic type of water which flows to the east and which is generally referred to as the Aleutian Current. As it nears the American coast one branch of this Aleutian Current turns south to form the California Current. Another branch turns north moving in a slow counter-clockwise gyral around the Gulf of Alaska. An extension from the head of the Gulf of Alaska which turns westward and then southwest, all the while undergoing acceleration, has been named the Alaskan Stream. Recent estimates of the volume transport of the gyral give values of 18 cubic kilometres per day, with surface velocities of 2 to 5 miles per day. It appears that the area of division of the west wind drift shifts from time to time to the south, the volume transport of the Alaska Gyral increases, and water warmer than usual moves northward past the Canadian coast. Many features of the oceanography of the North Pacific are related to the wind system which is variable in time and space. The dominant winds change with the season; the northwesterly winds favour upwelling on the coast, while southerly winds favour transport of surface waters towards the coast.

The Northeast Pacific Ocean is a prolific feeding ground for the various species of salmon that provide the valuable salmon fisheries on the Pacific Coast. The enrichment of the waters is provided through physical processes that bring the nutrients from the greater depths to levels where they enter into the cycle of production. These processes are enhanced in part by wind action. In this Northeast Pacific area in recent years, the successful application of oceanography to a major fisheries problem on an international scale has been ably demonstrated. The distribution of the salmon throughout this area, and the dependence of the North American coastal fishery on the well-being of the salmon in the sea have been demonstrated. This has emphasized the necessity of international control of fishing on the high seas.

Transportation problems on the Pacific Coast of Canada are confined to the immediate coastal areas, while the defence problems are those common to all coasts.

There are a number of problems of deep ocean circulation that are of fundamental importance to world well-being. The number of years during which a particle of water has been removed from contact with the atmosphere can be determined today for example by measuring the relative concentrations of carbon-14 and carbon-12 in a sample of sea water. The disposal of radioactive wastes, whether by burying on land or by dumping in the sea, constitutes a real problem which is being given attention by international bodies. The disposal of such wastes has been carried out today without too much consideration to the possible end result. Eventually, whether buried on land or dumped in the sea, these materials will be carried about in the great circulation systems of the oceans. The time factor is of course the major concern.

The sun provides the energy to move both sea water and air between the "furnace" of the tropics to the "condensers" of the polar regions. The circulation of the working fluids is demonstrated by the wind systems and the currents of the sea. In the circulation system of the oceans, the equatorial currents and the other major currents, such as the Gulf Stream and Kuroshio, are well known. The vertical circulations and the return currents at the abysmal depths are however at present only matters of very rough estimates. The rate of transport of deep water between high and low latitudes and the rate of exchange of heat with surface water in high latitudes determine the total gain or loss of heat by the oceans. The time element may be a year, a decade or centuries. Hence a very small temperature change in the deep waters would be indicative of a departure from a radiation balance between the sun and the earth. This radiation balance is a prime parameter in the problem of long-range weather forecasting.

The practical end result of productivity in the sea is measured by man in the amount of food he actually takes from it. In this sense we can look upon the areas of Canadian fisheries as most productive. At the same time, we are by no means harvesting the total protein crop available to us. We are aware, however, that there are fluctuations in the abundance of some of the more prized species of fish and animal life, and we are reaching the point in some areas and in some species that man takes all that can be readily caught. We can, therefore, expect that in some species there will be greater fluctuations in abundance, unless the particular fishery is brought under proper management. It is fundamental that knowledge of the factors affecting the production of stocks is necessary to proper management. Fundamentally, productivity has a much broader meaning. The food cycle in the sea involves the process of photosynthesis wherein plants grow under the action of sunlight. The great bulk of this primary production exists in the seas in the form of phytoplankton, and it is estimated that the amount produced per year exceeds the production of living matter on land. In the complete food chain from the minute plants to the full-grown fish and animal life there are many weak links or "limiting factors". The amount of sunlight available for plant production and growth varies with latitude, time of year, state of the sky and sea

surface, and qualities of the water. The necessary nutrient materials vary in quantity and availability. As all growth is a chemical process, temperature is a factor in the rate of production. The food chain is broken down at times by the ferocious grazing of living organisms on plant life or by one organism on another. For these reasons, the productivity is more accurately measured by the actual determination of the decrease of carbonate or the increase of dissolved oxygen in a sample of sea water where photosynthesis is taking place. The supply of nutrients, particularly those containing nitrogen and phosphorus, is renewed by the decomposition of dead plants and animals through the action of bacteria. The factors affecting the bacteria and other agencies of decay, particularly at the mud–water interface at the bottom of the sea, are not very well known.

As on land, if the end product is not harvested, it dies and returns to the cycle of the sea. A population explosion or an excessive schooling of fish may overgraze a portion of the sea, or may deplete the oxygen, and hasten its removal from the living chain. Another type of population explosion is involved in the phenomenon of the red tide, which proves toxic to higher forms of life in the sea. The destruction of the tile fish in the Gulf of Maine in the late nineteenth century, and the destruction of an estimated millions of tons of fish in the Indian Ocean in 1959 are examples of the effects of sharp changes in environment. That disease has killed off bodies of fish has been well demonstrated in the disasters that have affected Atlantic Coast oyster areas, and by observations over a period of years on Atlantic herring.

The geological history of the oceans is, to a large extent, recorded in the sediment of the deep ocean basins. The processes of sedimentation are associated, in part, with the deep ocean circulation, one phase of which may involve turbidity currents of considerable magnitude. During the present year, man has conquered the outer shell of "inner space" by means of the bathyscaphe. This new tool may now offer extraordinary facilities for penetrating the earth's crust to depths only dreamed of to date by geophysicists. We know that a great diversity characterizes the floor of the ocean basins and in the general plan we can distinguish between abysmal and shallow depths, shoals, ridges, and canyons. In detail, a knowledge of the ocean floors would offer insight into the building and erosion processes which we might consider at least in some areas as more accelerated than those on land. Mountain ranges there are under the surface of the sea mightier than those we know of on land. In the investigations of "inner space" the geophysicist has a particular interest in comparing his theories of mountain building as between the continents and the oceans.

General Oceanographic Activities 1957–60

During the period reported upon, Canada contributed to the oceanorgraphic projects of the International Geophysical year (IGY). The projects involved were Sea Level Recording, Deep Water Circulation, Polar Front Survey, Multiple Ship Surveys, Observations from Weatherships, and Ice Island Observations.

The study of tidal phenomena and mean sea level behaviour on our east, west and Arctic coasts was carried out by the Canadian Hydrographic Service. In the first two regions there existed a reasonably good network of permanent tide gauging stations from which basic data have been obtained over a long period of years. A concerted effort was made to rectify a deficiency of information in the Arctic and sub-Arctic regions. Two permanent stations were established, one at Resolute Bay and another at Brevoort Harbour. In addition, a series of temporary stations were established along the coasts of Baffin Island, Hudson and James Bays. The tidal station at Brevoort has since been closed and a new one opened up at Tuktoyaktuk on the Beaufort Sea. Preliminary work has been carried out at several other sites in the Arctic under a program for a network of permanent tide gauges in northern Canada. A regular series of oceanographic stations for monitoring the densities of the water column were maintained on the east and west coasts throughout the IGY period by the Fisheries Research Board and the Defence Research Board.

Contributing to the study of deep ocean water circulation, as programmed on an ocean-wide basis for IGY, an extensive survey was made of the waters of the western North Atlantic between Bermuda and Davis Strait. The sea program included observations of temperatures and salinities, and chemical analysis for dissolved oxygen, phosphate, silicate and nitrite. Various biological collections were also made. While bottom water conditions were fairly uniform in the Atlantic Ocean basins with salinities and temperatures holding to 34.9‰ and 2.5°C, surface salinities and temperatures ranged from 36.5‰ and 27.0°C off Bermuda to 31.5‰ and 4.0°C in Baffin Bay.

Extension of seasonal cruises involved the Grand Banks and the area to the east of Newfoundland in March and October, 1958, and in February and August, 1959. Oceanographic conditions on the Scotian Shelf were monitored seasonally in 1958 and 1959. The Labrador Current was again sectioned from Baffin Island southward during the summer of 1959. All of this contributed to the Polar Front Survey in the western North Atlantic which was co-ordinated through ICES and ICNAF.

An International Passamaquoddy Fisheries Board was set up in 1957 to investigate the effect of power dams in a proposed project for developing tidal power in the Passamaquoddy Region. The investigation under this Board consisted, in part, of an intensive effort over a three-year period to determine the tidal and non-tidal currents, the distribution of water properties and the controlling or relating factors. The study encompassed the Passamaquoddy Bay region and the contiguous regions of the Bay of Fundy and the Gulf of Maine. The field programs, which involved current measurements from an anchored ship, drift bottle releases, temperature and salinity observations, transport measurements by electromagnetic methods, and drift pole tracking have been completed. The analysis and interpretation of the data have been directed to estimating the changes that may occur if power dams are installed. The Fisheries Research Board and the Canadian Hydrographic Service co-operated in various phases of this project.

On the Atlantic Coast, the tendency is now toward lowered water temperatures. This tendency was particularly intensified during the later

months of 1958 and the winter months of 1959. That this trend has prevailed in more northerly areas in recent years is shown by a comparison of water conditions in the Labrador Sea and Davis Strait in the period 1920–35 with those of the period 1950–55. The decrease in water temperatures is of the order of one to four degrees centigrade in the surface waters, and one degree or less at 100 and 200 metres. In contrast, the bordering Atlantic waters were slightly warmer for the period 1950–55. Lowered salinities seem to be associated with lowered temperatures.

On the Pacific coast, contributing to both the Polar Front surveys and the Multiple Ship surveys, extensive coverage of the eastern North Pacific was made between July 1958 and December 1958. Additional coverage was made in 1959. These activities included quasi-synoptic surveys of the Aleutian area, the mid-ocean and coastal regions and the series observations at Ocean Weather Station "P" and along the coast. A continuing series of observations at Ocean Weather Station "P" was inaugurated, and in 1959 the section between the coast and the Station was occupied at approximately six-week intervals. Data are now on hand to define the predominant features of the water masses, the properties of the water, the structure and the currents in the upper 2000 metres of the eastern sub-Arctic Pacific. Attention has also been directed to progressive as well as seasonal changes in oceanographic conditions.

The recent studies on the Pacific coast in relation to the Northeast Pacific have shown that over the area generally precipitation exceeds evaporation. The resulting fresher surface water moves through and out of the region entraining sea water from below. A halocline separates an upper layer of comparatively uniform salinity from a more saline lower layer. In the lower layer, two primary sub-surface water masses, differing in temperature, may be identified. As these waters rise through the halocline to depths of less than 100 metres, they lose their identity. Through 1957 and 1958 there has been an intrusion of a warm water mass northward past the Canadian coast. This intrusion is readily described at depths of 200 metres near the coast, and at depths of 100 metres in the center of the Gulf of Alaska. It reached its maximum in August 1958, and has since undergone numerous fluctuations in strength.

Productivity studies of the waters of the North Pacific have shown them to be rich in nutrient material. Phosphates and silicates are always abundant but there is some evidence that nitrates may become a "limiting factor" as in near-shore waters. Primary productivity is generally controlled by the amount of light. A study of primary productivity has been carried out at Ocean Weather Station "P". This involved the determination of the standing crop of phytoplankton and the factors that affect it, such as insolation, light penetration, nutrient content, horizontal and vertical water movements, temperature and salinity structures, and the heat budget. The upwelling that is associated with a cyclonic water movement, as exemplified in the Alaska Gyral, encourages a high productivity in the open waters of the Gulf of Alaska. Seasonally, a high productivity would be expected in the Canadian coastal areas, when surface waters are moved offshore by the prevaling northwesterly winds. There is a

suggestion that waters of comparatively low dissolved oxygen content move shoreward at such times and may be a limiting factor to some living organisms on the shelf.

Theoretical studies have developed methods of computing the potential wind-driven transport in the ocean from the distribution of barometric pressure. The methods have been applied to North Pacific waters, and monthly assessment charts are being issued.

Throughout this period reported upon, the Canadian Hydrographic Service has been associated with a number of national developments requiring detailed investigations of tides and currents. Among these were the studies relating to tidal power development, causeway construction across Northumberland Strait, design of harbour facilities on the east coast, currents through the Gulf Islands and Smith Sound, and features of the Ripple Rock project on the west coast.

Oceanographic investigations in the Arctic were again carried out in 1957 by the Atlantic Oceanographic Group from H.M.C.S. *Labrador* in Hudson Strait and Frobisher Bay, Lancaster Sound, Bellot Strait, Wellington Channel, Baffin Bay and Davis Strait. The most intensive and detailed effort was made in Bellot Strait. There was a successful co-ordination of program in physical and chemical oceanography, marine biology, and marine geology. An extensive marine geological survey involved the taking of 30 cores and 126 grab samples. In 1959 a two-month detailed survey of currents and water properties was made over the inner half of Hudson Strait by the Department of Mines and Technical Surveys in the M.V. *Theta*. At six stations along a cross-section currents were recorded continuously at a depth of 30 metres for seven weeks, while at each station currents were observed at several other depths for periods up to 72 hours. General oceanographic observations were made at each of these stations and also at intermediate points. The outer half of Hudson Strait was given attention in September–October of the same year by a team of oceanographers, with the *Labrador* (now the C.G.S. *Labrador*), involving the Department of Mines and Technical Surveys, the Fisheries Research Board, and the Great Lakes Geophysical Research Group. During 1959 too, the Arctic Unit of the Fisheries Research Board made a very thorough coverage of the southeastern portion of Hudson Bay as well as James Bay. Of particular interest to the oceanography of the Canadian eastern Arctic is the recently published paper on the annual oceanographic cycle at Igloolik. An oceanographic station was occupied over a full year in a region of net flow of Arctic water from Fury and Hecla Strait. Water temperatures varied only by 3.55°C at all depths during the year and only by 0.07°C between November and May. Salinities varied between 1.68‰ and 32.59‰. Oxygen content, inorganic phosphate, and plankton varied greatly throughout the period.

The general oceanography of Hudson Strait is now fairly well known particularly after the intensive surveys of 1955, 1956 and 1959. Waters from Foxe Basin, Hudson Bay, and the Atlantic Ocean enter the strait, and each water of contrasting characteristics exerts its own specific influence on conditions in the strait. The deep water circulation is characterized by two opposing flows, one originating from Hudson Bay and Foxe Basin, and the other originating from the Atlantic Ocean.

The extent of Atlantic water intrusion into the strait is dependent, in part, on the volume of discharge from Hudson Bay. The surface circulation of the strait is dominated by what appears to be a steady outflow from Hudson Bay along the southern side of the strait. During July 1957, the peak net discharge from Hudson Bay eastwards was calculated at 0.3 $\times$ 10^6 cubic metres per second. Later in the season, the calculated discharge amounted to 0.2 $\times$ 10^6 cubic metres per second.

On Ice Island T-3, where oceanographic observations over a period of five months were made in association with the Cambridge Research Center of the USAF in 1958, the average drift of the island was of the order of two miles per day. Water temperatures recorded varied between 0.47°C and -1.71°C below the ice, while temperatures at depths of 2,000 metres varied but little during the period of observation (-0.1°C at 1,000 metres; -0.4°C at 2,000 metres). At 150 metres, the phosphate content was exceptionally high with maximum values throughout the summer at 3.0 microgram-atoms per litre. Oxygen concentrations were generally high.

Under the expanding program of the Department of Mines and Technical Surveys, a Polar Continental Shelf Project was set up in 1959. This project involves integrated investigations under various disciplines in the general area of the polar continental shelf and the Arctic Ocean within the Canadian Archipelago. The study of oceanic waters of Arctic Canada and the adjacent Arctic Ocean form a major part of the project. The long-range objective of the oceanographic studies is to obtain an understanding of the distribution, extent, composition, movement and history of the oceanic waters lying to the north of the mainland of Canada. The field work under this project was initiated in 1959. For technical reasons the area involved has been divided into blocks about 350 kilometres wide and extending out to sea a distance of 500 kilometres. A block based on Ellef Ringnes Island serves as the initial area of investigation. The work here in 1959 was of an exploratory nature and involved problems of position fixing and of operation under the prevailing conditions on the ice. The field program in 1960 will continue until early October. Disciplines involved are marine geology, seismology, marine biology, gravity, magnetism, sea-ice studies, hydrography, surveying and oceanography. In addition to three traverses running out to sea at intervals of 150 kilometres, sections are being occupied across the various straits and passages within working distance of the base.

Working Group on Ice in Navigable Waters

The Working Group on Ice in Navigable Waters was set up under the Joint Committee on Oceanography (now the Canadian Committee on Oceanography) to co-ordinate and encourage research in the field of ice in navigable waters. Particular attention has been given to the following activities:

(a) The development of techniques for forecasting ice growth, degeneration and movement,

(b) Studies of the physical properties of ice,

(c) Studies of the dynamics of the ice–air and ice–water interfaces,
(d) Climatological studies,
(e) The promotion of a standard terminology and codes for reporting ice.

Canadian studies of the physical properties of sea ice have been furthered largely by the Ice Research Project of McGill University and the Defence Research Board's Northern Laboratory at Fort Churchill. The Ice Research Project was set up in 1956. A field station at Shippegan, N.B., was operated during the winter for the years 1956–59, where the work consisted mainly of beam tests, ring tensile tests, and the relation of ultimate strengths to other variables. Crystal size seemed to be a more important independent variable than salinity. Further ring tensile and shear tests were carried out in the Northern Laboratory of the Defence Research Board at Churchill. In 1959, elastic properties of ice were studied in Barrow Strait, Young's modulus was related to salinity, and an empirical formula developed. The composition of salt water ice is being studied at McGill. Ice observing and reconnaissance is being carried out by the Meteorological Branch of the Department of Transport on the east coast, as well as in the Arctic, with a staff of fourteen trained and experienced ice observers. An additional ten observers will be undergoing training this year. The service is particularly valuable to winter and spring shipping in the Gulf of St. Lawrence and to the supply services in the Arctic. The Geographical Branch of the Department of Mines and Technical Surveys has completed in co-operation with DRB and the RCAF five consecutive years of ice reconnaissance in the Gulf of St. Lawrence, the results being intended chiefly for historical and climatic purposes.

During 1959, the ice forecasting services were taken over by the Meteorological Branch of the Department of Transport and the Ice Central is now located at Halifax, N.S., with expanded supporting staff and facilities. Forecasting activities cover the shipping channels below Quebec City, the Strait of Belle Isle, the shipping route to Churchill in Hudson Bay and the re-supply lines to weather station and DEW line sites. Five-day and three-day forecasts are issued by the Ice Central. These were supplemented in some areas of the Eastern Arctic by two-day forecasts and advisories.

The National Research Council has been giving attention to energy exchanges from snow, ice and water surfaces, and has been studying frazil ice formations in the St. Lawrence River. The Ice Atlas of the Canadian Arctic prepared under a Defence Research Board contract is now being printed. The new Pilot of Arctic Canada, Vol. 1, has recently been published by the Canadian Hydrographic Service. A section of this Pilot deals with Arctic ice. Volume 2 will be issued this year.

Institute of Oceanography—University of British Columbia

Graduate studies in oceanography are attracting a small but increasing number of Canadian students. The graduates have been readily absorbed into various departments of the government research activities. The Institute is receiving additional financial support from the National

Research Council in the form of annual grants. The additional funds have been used to increase the staff of the Institute and to provide adequate research equipment and facilities. Research activities include the study of the distribution of water properties in British Columbia inlets, theoretical studies of internal waves, various aspects of wave/shear current interactions, turbulent mixing in a fluid in the presence of a density gradient, and the distribution of algae and of zooplankton as indicators of water mass characteristics and movements. Recent additions to staff will introduce further disciplines to the research program.

Institute of Oceanography—Dalhousie University

Funds for the establishment of the Institute of Oceanography at Dalhousie University were made available by the National Research Council in 1959, and support will be continued on an annual basis. The research program includes studies on the dynamics of zooplankton production, the cycle of energy in the surface of the sea, marine bacteriology especially at the sediment–water interface, and the age of sediments. The disciplines of physics and geology will enter into the research program as staff appointments are completed. Courses at the graduate level in oceanography are being offered.

The Great Lakes Geophysical Research Group

Several years of preliminary geophysical research in Lakes Erie, Huron and Ontario, and in Georgian Bay were furthered by the Ontario Department of Lands and Forests. This was followed by the organization of the Great Lakes Geophysical Research Group under the University of Toronto to give greater attention, in an expanded program, to the geophysical problems of our great inland seas. The expanded program is furthered in part by a grant from the National Research Council and through the provision of a ship by the RCN. Assistance in operating the ship has been received from the Federal Department of Mines and Technical Surveys and from the National Research Council. Particular attention is being paid to physical limnology and studies of low-level atmospheric conditions over the lakes. Studies of bottom sediments, of waves, and of shore erosion processes form part of the program. Graduate training in hydrometeorology is offered as well as courses in other phases of geophysics.

The Royal Society of Canada and Oceanography

The activities of the Royal Society of Canada in the field of oceanography extend over a period of seventy years, as it was in part, through Section III, that the Tidal and Current Survey was established. Sixty years ago the activities of Section V resulted in the establishment of a Marine Biological Station. During the last several years the interest of the Society in the field of oceanography has been furthered by a Committee on Oceanography. The Committee is comprised of representatives of Section III, IV and V and through its activities has continued to further the development of oceanography on a national scale.

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EXPERIMENTAL INFECTIONS OF CYCLOPID COPEPODS WITH TRIAENOPHORUS CRASSUS FOREL AND T. NODULOSUS (PALLAS)[1]

N. H. F. Watson and J. L. Price[2]

Abstract

Coracidia of both *Triaenophorus crassus* and *T. nodulosus* were fed to 19 species of cyclopid copepods, and coracidia of one or the other were fed to 4 other species. Procercoids of both species of parasite developed in nine species of cyclopids. Three species were infected with *T. crassus* only and four with *T. nodulosus* only. Size or feeding habits of the specimens did not influence the ingestion of coracidia. The presence of non-feeding individuals may have influenced the infection rate of some species. Herbivorous species, carnivorous species, and species which did not become infected ate large quantities of coracidia.

Microcyclops varicans rubellus was the most easily infected cyclopid species in the experiments. It is probably an important host of *Triaenophorus* in North American lakes along with *Cyclops bicuspidatus thomasi*, *Cyclops brevispinosus*, and, in certain areas, *Cyclops scutifer*.

Introduction

The first parasitic stage in the postembryonic development of pseudophyllidean tapeworms occurs when the free-swimming larva or coracidium is eaten by a copepod. The embryonic larva or onchosphere loses its ciliated coracidial covering, burrows through the gut wall of its host, and develops into a solid procercoid larva. This portion of the life cycle was worked out for *Diphyllobothrium latum* by Janicki and Rosen in 1918 (Rosen (10)), and the similar life history of *Triaenophorus nodulosus* (Pallas) was described by Rosen in 1919 (10). Michajlow (4) described the development of the eggs, coracidia, and procercoids of *T. crassus* Forel. According to Miller (6, 7), *Cyclops bicuspidatus thomasi* Forbes is the principal first intermediate host of *Triaenophorus* in North America. *C. strenuus* is considered the most effective natural host by European workers.

The present study was undertaken as part of the research into the ecology of *T. crassus* currently being carried out by the Fisheries Research Board of Canada. Nearly 30 years ago, Michajlow (5) showed that several European species of the family Cyclopidae could act as first intermediate hosts of *T. nodulosus*. He felt that the successful development of the procercoid in the body cavity of a given species of cyclopid depended largely upon two factors: whether the digestive action of the copepod killed some or all of the coracidia and whether the procercoid could develop normally in the milieu provided by the haemocoele of the cyclopid. On this basis he considered

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C. bicuspidatus Claus, which is closely related to our North American subspecies, of secondary importance as a host, as many of the coracidia eaten were digested.

Since several European species of cyclopids were potential first intermediate hosts of *Triaenophorus*, the investigation of several North American species of cyclopids was expected to prove that more than one species would be infected. This study attempts to evaluate the importance of each of these species as natural hosts by considering its degree of infection and ecological relationships within the parasite's life cycle.

Materials and Methods

The basic procedure undertaken in this study consisted of placing uninfected cyclopids in contact with viable coracidia and examining them at some later date for the presence of normal procercoids.

Triaenophorus eggs were obtained from adult worms found in the intestines of pike during May and early June. The eggs hatch into free-swimming coracidia a few days after they are removed from the worms if they are cultured at room temperature. The coracidia are collected by pipette from the surface of jars containing hatching eggs. Since they live only about 48 hours, the supply of fresh coracidia limited the number of infections which could be carried out. In order to obtain coracidia over a longer period of time, jars of eggs were refrigerated. When the water covering the eggs was changed regularly to provide aeration and remove excessive growths of protozoans, sufficient quantities of coracidia could be obtained for periods up to 3 weeks or a month. Subsamples of the cultures were removed from the refrigerator and held at room temperature overnight to induce hatching.

The cyclopids to be infected were collected from various localities, chiefly from the vicinity of Toronto, Ontario, and Heming Lake, Manitoba. Most of them were isolated, identified to species, classified by stage of development and sex, and maintained individually in 1-in.-sq. staining dishes. Coracidia were then added to the dishes containing the individual cyclopids. Each animal was examined for the presence of procercoids a week after introduction of coracidia. This time lapse allowed the procercoids to develop. Before examination the cyclopids were anaesthetized in a 0.1% solution of MS-222, an anaesthetic widely used in fishery biology. The procercoids continued to pulsate in the cyclopid's body after the host was rendered inert, and thus were detected more easily. The number of infected cyclopids and the number of procercoids per cyclopid was noted. These individually reared cyclopids were kept and re-examined from time to time until their death.

A slightly different method was used to obtain larger numbers of infected animals. This consisted of adding relatively large numbers of copepods to a jar or dish and exposing them to quantities of eggs and coracidia. The results obtained from these mass cultures were not as exact as those from the infection of individual specimens, especially as we were not able to estimate the number of copepods that had died before examination.

Although a few experiments of this type were carried out at Heming Lake in 1953 and subsequent years, their results were inconclusive. The major part of the work was undertaken by the junior author at the University of Toronto in 1957 and by the senior author at Heming Lake in 1958 and 1959.

Several experiments were carried out at Heming Lake in 1959 to determine the number of coracidia eaten by individuals of several species of cyclopids. In these experiments several cyclopids were introduced into a counting cell containing a known number of coracidia. After a given interval, usually 8 hours, but upon one occasion 2 days, the culture was anaesthetized, the cyclopids removed to separate dishes for future examination, and the number of uneaten coracidia recorded.

Nomenclature

The nomenclature used in describing the cyclopid copepods in this paper follows the general outline of that adopted by Yeatman (11, 12). The family Cyclopidae is divided into several genera. Species of eight of these, *Ectocyclops*, *Eucyclops*, *Macrocyclops*, *Mesocyclops*, *Orthocyclops*, *Paracyclops*, *Tropocyclops*, and *Cyclops*, are mentioned in this paper. Within the genus *Cyclops* as defined in this classification, there are several recognized groups of species. The species of the genus *Cyclops* mentioned in this paper may be placed in the following groups:

Microcyclops including *C.* (*M.*) *varicans rubellus* Lilljeborg
Acanthocyclops including *C.* (*A.*) *vernalis* Fischer
Acanthocyclops including *C.* (*A.*) *brevispinosus* Herrick
Acanthocyclops including *C.* (*A.*) *venustoides* Coker
Diacyclops including *C.* (*D.*) *bicuspidatus* Claus
Diacyclops including *C.* (*D.*) *bicuspidatus thomasi* Forbes
Diacyclops including *C.* (*D.*) *navus* Herrick
Cyclops s.s. including *C. scutifer* Sars
Cyclops s.s. including *C. strenuus* Fischer

These groups of species have been considered either subgenera or genera by different workers. Present opinion among North American authorities appears to favor subgeneric rank for these groups. However, the authors of this paper believe that the fifth foot structure and incomplete segmentation of the swimming feet of *Microcyclops* are of sufficient distinction to set this group off as a separate genus. This will be discussed more fully in future papers.

Price (9) has shown that *C. vernalis* Fischer as described by present taxonomic methods is in effect a species group consisting of at least seven reproductive isolates. One of these isolates fits the description of *C. brevispinosus* Herrick and should be referred to by that name. Tentatively, the others have been designated *C. vernalis* A, B, D, E, F, and G.

C. bicuspidatus thomasi Forbes is a common limnetic cyclopid of North American lakes often referred to as *C. bicuspidatus*, and occasionally as *C. thomasi*. It should not be confused with the Eurasian *C. bicuspidatus* Claus, which probably occurs occasionally in a few limited habitats in North America.

Results

During the course of the experimental infections, 23 species of cyclopids were exposed to coracidia. Large numbers of some of the more abundant species were used in the experiments, but only a few of some less-common species could be obtained. Nineteen species were exposed to coracidia of both *T. crassus* and *T. nodulosus*, three were exposed to *T. crassus* only, and one was exposed to *T. nodulosus* only. The number of individuals of each species exposed, the number infected, and the resultant percentage infection is shown in Table I for *T. crassus* and in Table II for *T. nodulosus*.

Cyclopids which were infected with one species of *Triaenophorus* were generally also infected with the other. Individuals of *Microcyclops varicans rubellus* were found to be infected most frequently with both species of *Triaenophorus*. Individuals of seven species of *Cyclops*, including *C. brevispinosus*, *C. bicuspidatus thomasi*, *C. navus*, and four reproductive isolates of *C. vernalis*, as well as *Mesocyclops edax*, were also infected with both parasites. *Macrocyclops albidus*, *M. fuscus*, and *Ectocyclops phaleratus* were infected with *T. crassus* but not with *T. nodulosus*, while *Eucyclops speratus* and two isolates of *Cyclops vernalis* were infected with *T. nodulosus* and not *T. crassus*. *Cyclops venustoides*, *Orthocyclops modestus*, *Tropocyclops prasinus*, and *Eucyclops agilis* were exposed to coracidia of both species without infection. *Cyclops scutifer* was exposed to *T. nodulosus* only and became infected, while *Mesocyclops leuckarti*, *Eucyclops prionophorus*, and *Paracyclops fimbriatus* were exposed to *T. crassus* coracidia only with negative results.

The procercoids which developed in the haemocoele of most species of infected cyclopids were similar and possessed typical cercomeres bearing the embryonic hooks. The procercoids found in *Eucyclops speratus* and *Ecto-*

TABLE I

The number of each species of cyclopid exposed to, and the number and percentage infected with, *Triaenophorus crassus* at Toronto and Heming Lake

Species	Toronto experiment			Heming experiment			Total			Rank
	Exposed	Infected	%	Exposed	Infected	%	Exposed	Infected	%	
Microcyclops varicans rubellus	39	35	89	13	8	62	54	43	80	1
Cyclops vernalis E	16	12	75	—	—	—	16	12	75	2
Cyclops navus	17	14	82	2	0	—	19	14	74	3
Cyclops vernalis G	46	22	48	—	—	—	46	22	48	4
Ectocyclops phaleratus	2	1	50	3	1*	33	5	2	40	5
Cyclops vernalis F	35	10	29	—	—	—	35	10	29	6
Cyclops bicuspidatus thomasi	50	11	22	184	55	30	234	66	28	7
Mesocyclops edax	61	16	26	45	0	—	106	16	14	8
Macrocyclops fuscus	33	3	9	6	0	—	39	3	8	9
Cyclops vernalis A	46	3	7	—	—	—	46	3	7	10
Cyclops brevispinosus	10	0	—	28	2	7	38	2	5	11
Macrocyclops albidus	24	1	4	6	0	—	30	1	3	12
Cyclops vernalis B	20	0	—	—	—	—	20	0	—	
Cyclops vernalis D	31	0	—	—	—	—	31	0	—	
Cyclops venustoides	14	0	—	—	—	—	14	0	—	
Mesocyclops leuckarti	15	0	—	—	—	—	15	0	—	
Eucyclops speratus	18	0	—	29	0	—	47	0	—	
Eucyclops agilis	14	0	—	25	0	—	39	0	—	
Eucyclops prionophorus	—	—	—	12	0	—	12	0	—	
Paracyclops fimbriatus	—	—	—	14	0	—	14	0	—	
Tropocyclops prasinus	—	—	—	22	0	—	22	0	—	
Orthocyclops modestus	—	—	—	13	0	—	13	0	—	

*Procercoids in these infected cyclopids were abnormal and lacked cercomeres.

cyclops phaleratus did not possess a cercomere and therefore are regarded as atypical. It is not known whether these would have infected the next intermediate host.

The results of the infection of several mass cultures are included in Tables I and II. The mass cultures were carried out to infect quantities of *C. bicuspidatus thomasi* and *C. brevispinosus* because of the relative ease with which fairly large numbers of infected cyclopids could be obtained. While the results may not be exact, they are comparable and were therefore included.

TABLE II

The number of each species of cyclopid exposed to, and the number and percentage infected with, *Triaenophorus nodulosus* at Toronto and Heming Lake

Species	Toronto experiment			Heming experiment			Total			Rank
	Exposed	Infected	%	Exposed	Infected	%	Exposed	Infected	%	
Microcyclops varicans rubellus	23	19	83	—	—	—	23	19	83	1
Cyclops vernalis E	22	16	70	—	—	—	22	16	70	2
Cyclops vernalis G	55	35	60	—	—	—	55	35	60	3
Cyclops bicuspidatus thomasi	77	23	30	123	53	44	200	76	38	4
Cyclops navus	36	11	31	—	—	—	36	11	31	5
Cyclops vernalis F	27	8	29	—	—	—	27	8	28	6
Cyclops brevispinosus	11	3	27	36	9	25	47	12	26	7
Cyclops vernalis B	19	3	16	—	—	—	19	3	16	8
Cyclops scutifer	13	2	15	—	—	—	13	2	15	9
Cyclops vernalis A	47	5	11	—	—	—	47	5	11	10
Mesocyclops edax	18	4	22	28	0	—	46	4	9	11
Cyclops vernalis D	55	2	4	—	—	—	55	2	4	12
Eucyclops speratus	—	—	—	46	1*	2	46	1	2	13
Eucyclops agilis	—	—	—	15	0	—	15	0	—	
Cyclops venustoides	10	0	—	—	—	—	10	0	—	
Macrocyclops albidus	13	0	—	2	0	—	15	0	—	
Macrocyclops fuscus	4	0	—	—	—	—	4	0	—	
Tropocyclops prasinus	—	—	—	10	0	—	10	0	—	
Orthocyclops modestus	—	—	—	12	0	—	12	0	—	
Ectocyclops phaleratus	—	—	—	3	0	—	3	0	—	

*Procercoids in these infected cyclopids were abnormal and lacked cercomeres.

A summary of the individual infections by developmental stages (Table III) indicates that adult *C. bicuspidatus thomasi* are more easily infected with both parasites than immatures (fourth and fifth copepodite stages). Although samples of other species are not large enough to permit individual examination, the pooled data show the same relationship. Adult male *C. bicuspidatus thomasi* appear to be more readily infected by both parasites than either

TABLE III

Rate of infection of immature and adult cyclopids with *Triaenophorus crassus* and *T. nodulosus* (number of cyclopids in parenthesis)

	iv copepodite, %	v copepodite, %	Adult male, %	Adult female, %	Total adult, %
T. crassus					
C. bicuspidatus thomasi (Toronto and Heming data)	20.0 (70)	18.0 (39)	64.0 (11)	59.0 (17)	58.8 (28)
All species combined (except *C. b. thomasi*) (Toronto data)	**19.4 (62)**	**23.3 (60)**	**43.8 (89)**	**29.4 (177)**	**34.2 (266)**
T. nodulosus					
C. bicuspidatus thomasi (Toronto and Heming data)	7.4 (27)	29.3 (17)	85.0 (12)	29.0 (21)	49.0 (33)
All species combined (except *C. b. thomasi*) (Toronto data)	29.6 (27)	53.3 (15)	43.7 (103)	25.8 (182)	32.3 (285)

females or immature forms, and the fifth copepodite stage appears to be less resistant to infection (at least by *T. nodulosus*) than the fourth. Large quantities of yolky masses in adult females masked the presence of procercoids and a few infected individuals may have been overlooked on examination. No such obstacle existed to observation of males or immature individuals.

In the experiments, more than 1 procercoid developed in many of the animals; up to 10 procercoids were counted in some cases. The average number of procercoids per host was determined for several species at Toronto and Heming Lake, and is recorded in Table IV. Specimens of *Microcyclops varicans rubellus* support fewer procercoids per infected animal than do the specimens of the other larger species to which they are compared. This is undoubtedly the result of the smaller space available in the haemocoele of this species in comparison with that in the other species. The higher average of procercoids present in the Toronto experiments probably indicates that more coracidia were available to the infected cyclopids compared with the number used at Heming Lake. This is confirmed by a comparison with infections of cyclopids from nature where the numbers of coracidia present in the vicinity of a cyclopid are probably still lower. It has been the senior author's experience at Heming Lake that infected specimens from plankton tows almost invariably contain but one procercoid. Miller (6) has published similar results for Lesser Slave Lake, Alberta.

TABLE IV

The average number of procercoids of *Triaenophorus crassus* and *T. nodulosus* per infected cyclopid in the Toronto and Heming Lake experiments

Species	*T. crassus*, procercoids per infected animal		*T. nodulosus*, procercoids per infected animal	
	Toronto	Heming	Toronto	Heming
Cyclops bicuspidatus thomasi	5.4	3.5	2.9	1.4
Cyclops navus	5.4	—	1.9	—
Mesocyclops edax	4.9	—	—	—
Cyclops vernalis G	4.5	—	2.4	—
Cyclops vernalis E	3.3	—	1.9	—
Microcyclops varicans rubellus	2.6	2.0	1.9	—

Procercoids do not seem to die in the body cavity of infected hosts, nor do moderate numbers of them seem to affect the longevity of the host. The junior author determined the average number of procercoids per animal after infection and 30 days later, for individuals of several species, and found that while some of the cyclopids had died, the average number of procercoids per animal remained about the same. Infected cyclopids containing up to 10 procercoids have been kept for periods up to a month and a half before the cyclopid died. This approximates the maximum life span of uninfected animals under similar conditions. In all cases the number of procercoids present at the time of death was the same as when the cyclopid was first infected. Similar results were obtained by Miller (6) in his experimental infection.

Procercoids have been killed in living cyclopids after the animals were held for a short time at high temperatures. This was accidentally discovered when infected *M. edax* were found on subsequent examination to have lost their procercoids. Further trials showed that exposure to a temperature of 35° C for 5 minutes was sufficient to cause the death and subsequent dissolution of the procercoids in the few specimens available for experimentation.

A few experiments were performed to determine the number of coracidia eaten by cyclopids. Michajlow (5) demonstrated that large numbers of coracidia were eaten by all the cyclopids and diaptomids which he examined. This experiment attempted to disclose whether cyclopids which did not become infected ate coracidia.

Only a small number of experiments were carried out since sufficient quantities of coracidia were not available for a full-scale investigation. The eggs from which the coracidia were hatched had been stored under refrigeration for over a month and did not hatch too readily. The infection rate of the surviving cyclopids was much below that of the other experiments, so the coracidia may also have been less viable than those hatched earlier in the season and used for the remainder of the infections.

Individuals of all species tested ate coracidia (Table V). Results of the experiments do not indicate whether interspecific differences in the number of coracidia eaten exisr, or whether the differences in the number of coracidia eaten in different tests were due to availability of coracidia, number of cyclopids per culture, or any other possible causes.

In the test with *Mesocyclops edax* about a fifth of the coracidia remaining after the cyclopids were removed were dead and distorted. It appears that these coracidia were destroyed by the cyclopids. They were probably ingested and regurgitated or egested without complete digestion.

TABLE V

Number of coracidia of *Triaenophorus crassus* and *T. nodulosus* eaten after 8 hours and resultant infections in experiments carried out at Heming Lake, July 1959

Species	Description of cyclopids introduced	No. of coracidia			No. of *Cyclops*	
		Introduced	Eaten/ cyclopid	% introduced eaten	Alive week later	Infected
T. crassus						
C. b. thomasi	5 mature ♀	54	9.0	83.3	4	1
C. b. thomasi	5 mature ♀	51	8.0	78.4	4	0
C. b. thomasi	5 mature ♀	51	5.2	51.0	2	0
C. b. thomasi	5 mature ♀	52	7.2	69.2	1	0
C. b. thomasi	15 mature ♀	240	13.0	81.3	0	—
C. b. thomasi	10 mature ♀	104	7.8	75.0	8	0
C. b. thomasi	4 mature ♀	61	9.8	64.5	3	1
C. b. thomasi	10 mature ♂	70	6.6	94.3	2	0
C. b. thomasi	1 iv copepodite	53	20.0	37.7	1	0
M. varicans rubellus	8 mature ♀	65	7.1	87.6	6	0
M. varicans rubellus	8 mature ♀	69	8.1	95.3	6	0
C. brevispinosus	7 mature ♀	129	16.7	98.2	5	0
C. brevispinosus	1 iii copepodite	58	14.0	24.1	1	0
M. edax	6 mature ♀	69	5.5	47.8	5	0
M. edax	6 mature ♀	97	12.3	76.9	2	0
E. agilis	6 mature ♀	72	11.7	97.5	4	0
E. agilis	6 mature ♀	60	9.7	97.0	0	—
T. nodulosus						
C. b. thomasi	5 mature ♀*	51	10.2	100.0	5	0
C. b. thomasi	5 mature ♀*	52	10.4	100.0	5	1

*These cyclopids remained in the counting cells with the coracidia for 48 hours.

No marked differences were noted between the number of coracidia eaten by small and large species or between species classed as herbivorous and carnivorous. Both the smallest cyclopids, *Microcyclops varicans rubellus*, and the largest cyclopids tested, *Mesocyclops edax*, ate considerable numbers of coracidia. *Microcyclops varicans rubellus* and *Eucyclops agilis*, which are both considered to be herbivores, ate coracidia as did *Cyclops bicuspidatus thomasi*, *C. brevispinosus*, and *Mesocyclops edax*, which are considered to be carnivores.

If the number of procercoids which developed from these exposures to coracidia is normal, these experiments also indicate that large numbers of coracidia may have to be eaten for infection to occur. It is felt that some of the coracidia may have been only partially viable because the eggs from which they hatched had been held for abnormally long periods of time, so that the number actually required may be less than what is implied by these experiments.

Discussion

Nine species of cyclopids tested in these studies were capable of acting as hosts for both *Triaenophorus crassus* and *T. nodulosus*. Six others were infected upon occasion with either one or the other of the two parasites. These results confirm Michajlow's (5) conclusion that several species of cyclopids would serve as hosts for *T. nodulosus*, some being more suitable than others. In addition, it seems possible from our data to state that *T. crassus* and *T. nodulosus* react in a similar manner to their first intermediate hosts.

Under standard conditions, a measure of the susceptibility of a cyclopid species would be the proportion of individuals infected on exposure to either or both species of *Triaenophorus*. The experiments described in this paper indicate that some cyclopids are more susceptible to infection than others. From these results it appears that *Microcyclops varicans rubellus* is the most suitable host for *Triaenophorus*. Other suitable hosts are found within the subgenera *Acanthocyclops*, *Diacyclops*, and *Cyclops* proper of the genus *Cyclops*. Cyclopids in the genera *Mesocyclops*, *Macrocyclops*, *Eucyclops*, and *Ectocyclops*, and some species of the *Cyclops vernalis* group apparently are less favorable hosts, while those of the genera *Tropocyclops*, *Paracyclops*, and *Orthocyclops*, as well as *Cyclops venustoides*, are even less suitable as hosts.

Failure of some cyclopids to ingest coracidia might result if the coracidia were too small (or too large) to be eaten readily, or if they belonged to a class of organisms which was not generally chosen as food. Any physiological state which would curtail the cyclopid's food intake would also temporarily prevent ingestion of coracidia.

In the present experiment, both large (*Mesocyclops edax*) and small (*Microcyclops v. rubellus*) cyclopids ate coracidia (Table V). There was no apparent relationship between the number of coracidia eaten and the size of the cyclopids in our tests. Ingestion experiments, using various numbers of coracidia and more cyclopids in the small and large size range, are necessary to clarify this point.

Cyclopids have been classified by several authors, including Fryer (3), as either carnivorous or herbivorous in their food habits. In this experiment, both herbivorous and carnivorous species ate coracidia. This is substantiated by the observation that herbivorous species such as *Eucyclops agilis* could be reared on cultures rich in protozoans. These species are possibly better described as omnivores rather than herbivores. It is impossible to say that these forms will not eat coracidia simply because their normal food preference is for plant material.

The junior author has found that individual cyclopids may cease feeding for short periods of time during various periods in the life history. Feeding is stopped, for instance, before and during molts, during copulation, and possibly at other times as well. Other workers have found that certain stages of the life history of many cyclopids are quiescent non-feeding resting stages (Elgmork (2)). If the individuals in our experiments were in any of these non-feeding phases for the short while coracidia were present, they would not ingest coracidia. Our results do not indicate how much this factor may have affected the ingestion of coracidia and the subsequent infection data, but it may be responsible for the differences in infection between developmental stages of cyclopids, especially *Cyclops bicuspidatus thomasi*. Cole (1) reports a resting stage in the fourth copepodite stage of this cyclopid. Oil droplets in the body cavity and apparently empty gut cavities, which characterize this condition, were noted in some of the fourth copepodite, *C. b. thomasi*, examined at Heming Lake. While it is impossible from the data to state the duration of this condition in our fourth copepodite animals, it probably influenced the results of the experimental infections.

Once coracidia are ingested some may be digested. Michajlow (5) found that coracidia were digested by many of the species of cyclopids which he tested. In our experiments there was no direct evidence that digestion of coracidia took place. However, as noted earlier, some destroyed coracidia which may have been partially digested were found after they had been introduced to a culture of *Mesocyclops edax*.

The development of the procercoid in the haemocoele does not always proceed successfully. In some cases aberrant procercoids were found. It is possible that others may have developed, died, and disappeared before the cyclopids were examined.

Since several species can serve at least experimenatlly as hosts for *T. crassus* and *T. nodulosus*, an attempt will be made to evaluate their potentialities as hosts in nature. The suitability of a species as a host depends not only on its susceptibility to infection, but also on its presence in areas where coracidia and the next host in the life cycle are found. These factors may be conveniently considered in terms of geographic range, abundance, and habitat preference. Data on the known range, habitat, and local abundance were collected from several sources, including Yeatman (11, 12) and Pennak (8), and were combined with our infection results to obtain a measure of their probable importance as natural hosts (Table VI).

TABLE VI

The importance of experimentally infected species as natural hosts of *Triaenophorus crassus* and *T. nodulosus*

Species	Habitat and habits	Relative abundance	Susceptibility	Importance as host
M. varicans rubellus	A pond species, littoral and non-planktonic, widespread	Never found by the authors in quantity	Excellent	Unknown, may be important
C. vernalis E and G	Pond species, non-planktonic, local	Probably rare	Very good	Not well fitted
C. navus	Pond, littoral species, widespread	Uncommon	Moderate	Not well fitted
C. vernalis F	Littoral, non-planktonic, permanent ponds, lakes. Local	Probably rare	Moderate	Not well fitted
C. scutifer	Limnetic, in cold northern waters. Widespread	Common to abundant	Moderate to poor	Potentially important
C. brevispinosus	Limnetic, occurring in shallows as well. Widespread	Common to abundant	Moderate to poor	Possibly important
C. bicuspidatus thomasi	Limnetic, occurring in shallows as well. Widespread	Abundant	Moderate	The most important
C. vernalis A, B, D	Littoral, in permanent and temporary water. Local	Unknown	Poor	Not important
M. edax	Limnetic, widespread	Common	Poor	Probably not important
Macrocyclops sp.	Littoral and in pools. Widespread	Common	Poor	Not important
Eucyclops sp.	Littoral, widespread	Abundant	Developed aberrant procercoids	Not important
Ectocyclops phaleratus	In vegetation, widespread	Rare	Developed aberrant procercoids	Not important

Of the most completely susceptible cyclopids apparently only *Microcyclops varicans rubellus* could be more than locally important as a vector. Of the moderately susceptible species, *Cyclops scutifer*, *C. bicuspidatus thomasi*, and *C. brevispinosus* are probably the only ones widely enough distributed and abundant enough to act as first intermediate hosts of *Triaenophorus crassus* and *T. nodulosus* throughout the range of these species. The majority of naturally occurring infections at Heming Lake, which lacks *C. scutifer*, occur in *C. bicuspidatus thomasi* although a few have been recorded from *C. brevispinosus*. None of the lightly infected species are likely to act as first intermediate hosts in nature, except locally, because so few of them become infected. Of these, *Mesocyclops edax* is potentially the best suited as a host because of abundance and widespread range.

Summary

(1) Nineteen species of cyclopids were exposed to coracidia of *Triaenophorus crassus* and *T. nodulosus*. Three were exposed to *T. crassus* only and one to *T. nodulosus* only. Procercoids of both parasites developed in nine species, including *Microcyclops varicans rubellus*, four reproductive isolates of the *C. vernalis* group, *C. brevispinosus*, *C. bicuspidatus thomasi*, *C. navus*, and *Mesocyclops edax*. *Macrocyclops albidus*, *M. fuscus*, and *Ectocyclops phaleratus* were infected with *T. crassus* and not *T. nodulosus*, while *Eucyclops speratus* and two isolates of *C. vernalis* were infected with *T. nodulosus*. *C. scutifer*, which was not exposed to *T. crassus*, was infected with *T. nodulosus*. *Eucyclops agilis*, *E. prionophorus*, *Cyclops venustoides*, *Tropocyclops prasinus*, *Orthocyclops modestus*, *Paracyclops fimbriatus*, and *Mesocyclops leuckarti* were not infected.

(2) Differences in the proportion of cyclopids infected were noted between animals of different degrees of maturity and between sexes.

(3) Most experimental infections were heavier than natural ones and the average number of procercoids per infected animal varied directly as the size of the species involved.

(4) Procercoids live throughout the life of the infected cyclopid and do not seem to shorten the host's life appreciably. Exposure to high temperatures of the order of 35° C for a short time was found to cause the death and disappearance of procercoids in living *M. edax*.

(5) Large size and herbivorous food habits do not seem to prevent the ingestion of coracidia by the species of cyclopids examined and apparently large numbers of coracidia must be eaten for the development of procercoids in *C. bicuspidatus thomasi*.

(6) A review of the distribution, habitat, and infective properties of the species examined indicates that *C. bicuspidatus thomasi* and probably *C. brevispinosus* and *C. scutifer* are potentially the most important natural intermediate hosts of the two species of *Triaenophorus* examined. There is no reason why *Microcyclops varicans rubellus* should not also be an important natural host.

Acknowledgments

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SHELLFISH POISONING—ANOTHER NORTH AMERICAN GHOST*

J. C. MEDCOF,† *St. Andrews, New Brunswick*

THE TITLE of this article is borrowed from a Fredericton naturalist, Bruce S. Wright, for "The Ghost of North America" was the title of his recently published book on the eastern panther. There are interesting parallels in the histories of shellfish poison and of panthers in our part of the world. Both seem to have been here always, and people living in the out-of-the-way places where panthers and poison occur have extensive practical knowledge about them. However, these people are not usually writers, so records of their experiences are scarce. Indeed, I have chosen my title quite deliberately to impress you with the scarcity and elusiveness of reliable information on our poison problem. It is not easy to trace its history.

FIRST REPORTED CANADIAN POISONING

I would like to quote an extract from what I believe is the first published account of shellfish poisoning in Canada.[1] This was written on June 17, 1793, by Mr. Menzies (naturalist and surgeon on the voyage of Captain George Vancouver) and concerned the activities of two small-boat parties sent from the main vessels to explore what is now Mathieson Channel on the coast of British Columbia.

"Near the head of this arm they stopped to breakfast on the morning of the 15th where the people finding some good looking mussels about the rocks and shores, boiled a quantity of them . . . . but unfortunately for them . . . . these mussels proved to be of a deleterious quality as all those who had ate of them in any quantity were, soon after they embarked, seized with sickness, numbness about the mouth, face and arms, which soon spread over the whole body accompanied with giddiness and general lassitude; this was the case with three of the crew of the *Discovery's* boat . . . . One of them, John Carter, puked a good deal, and found himself so much relieved by it that he kept pulling on his oar till about one o'clock when the whole party stopped to dine; but in attempting to get out of the boat he was so weak and giddy that he fell down and he and the other two were obliged to be carried to shore. On this, Mr. Johnstone instantly directed a fire to be kindled and plenty of warm water to be got ready as soon as possible, that each of them might drink a sufficient quantity of it to operate as an emetic . . . . but, before it could be got ready, John Carter became . . . . very ill . . . . his pulse becoming weaker and weaker, his mouth and lips appearing black and his face and neck becoming much swelled together with faintness, general numbness and tremor. Under these circumstances he gradually sank without much struggle and expired just as soon as they were offering him the first draught of warm water which he was unable to swallow, and this sad affair happened within five hours from the time of his eating the mussels."

Captain Vancouver's own writings[1] continue the account as follows:

"They stopped in a bay for the night, where they buried the dead body. To this bay I gave the name, Carter's Bay, after this poor unfortunate fellow; it is situated in latitude 52°48′, longitude 231°42′, and to distinguish the fatal spot where the mussels were eaten, I have called it Poison Cove, and the branch leading to it, Mussel Canal."

These names given by Captain Vancouver are to be found even on our present-day charts of the British Columbia coast.

EAST-COAST POISON AND CONTROL PROBLEMS

You have heard enough about the west coast! Now let me show you our ghost's east-coast stamping grounds (Fig. 1). They are all in two districts —first, the estuary of the St. Lawrence[2] (north and south shores) and second, the lower Bay of Fundy.[3] I have identified three types of areas in each district —those where shellfish sometimes contain poison, as shown from study of shellfish extracts; those where illnesses have occurred, following consumption of local shellfish, and those where there have been deaths from this cause. Some of the reports which I have summarized in this figure date back to the period 1800-1810. But from here on I shall discuss the poison problem only as we have known it in recent years in New Brunswick sections of the Bay of Fundy district.

The last rumbling we heard from our ghost in the Fundy area was in 1958. That year there was one case of illness. A man was hospitalized in Saint

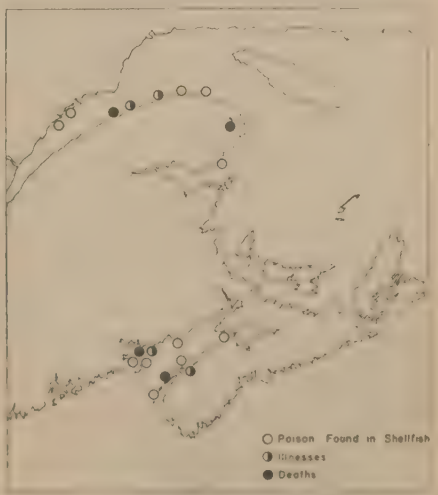

Fig. 1.—Eastern Canada showing the two districts affected by paralytic shellfish poison.

*Address to the New Brunswick and Prince Edward Island Branch of the Canadian Public Health Association, Fredericton, N.B., April 30, 1959.

†Head, Molluscan Shellfish Investigations, Fisheries Research Board of Canada, Biological Station, St. Andrews, N.B.

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John. In 1957, however, our ghost really walked.[4] Over one week-end, 27 New Brunswickers took sick for periods ranging from a few hours to nearly three weeks. Twelve years before that, in 1945, there had been another burst of illnesses.[3] A smaller group of New Brunswickers ate the same varieties of shellfish from the same places at about the same time of year and this brought on what we believe was the first east-coast outbreak of shellfish poisoning that could properly be called an epidemic. This 1945 epidemic created a real stir.

Just at that time there was a search under way to discover and study the marine organism responsible for the poison that had been found for a month or two every summer in shellfish in certain parts of Charlotte County, N.B. This search was being conducted by an unpaid volunteer worker at the Fisheries Research Board's Biological Station at St. Andrews, N.B. That worker was Mrs. Needler. The Fisheries Research Board provided her work space in the station laboratory and she got encouragement from the Department of Fisheries and from the Department of National Health and Welfare. The last two agencies were co-operating with other members of our station staff in studying other aspects of the poison problem.

Mrs. Needler's work was extraordinarily successful.[5] She found a one-celled flagellated plant that appeared in the plankton about the time the summer water temperatures approached their peak and disappeared soon after. The time of occurrence of this plant coincided exactly with the time when poison was present in the shellfish. This discovery fitted in with and explained many of the findings that others of our team were making. We had found a "key station" where the shellfish nearly always became poisonous earlier than elsewhere. We decided to use this in the same way our Defence Department would use the DEW line. The three government agencies agreed to maintain a regular sampling of shellfish at this key station, to close down commercial harvesting of clams (*Mya*) when poison appeared in them and to keep the clam-producing areas under quarantine until the danger passed. Clams were the only commercially important species of shellfish that were affected.

Everyone seemed satisfied with this control system once it was established. It was expensive to maintain but it permitted almost full commercial exploitation of our clam resources and afforded good protection to consumers of the commercially marketed products. Besides this, the Department of Fisheries posted signs during the danger periods, warning tourists, picnickers and other non-commercial clam diggers, who might be inclined to go gathering dangerously poisonous shellfish. Thus the clam industry prospered and all clam consumers were protected or at least warned of the risks of poisoning. Most people thought there was no need to continue investigations when we had such a good control system. And it was good—better than in most countries, because we had been able to work out the pattern of the annual recurrences of the poison. So work of the type Mrs. Needler had been doing stopped but routine control work went on smoothly year after year.

Canada
Department of Fisheries

WARNING
SHELLFISH AREA
CLOSED

As the Department of National Health and Welfare has determined that clams taken from

.....

area are affected with paralytic poison, this area is closed for the taking of clams except for use as bait or for treatment by the canning process; and is closed for the taking of mussels for human consumption.

G. R. CLARK,
Deputy Minister of Fisheries.

Notice Posted

.....19.....

.....
(Fishery Officer)

Anyone found guilty of defacing this poster will be prosecuted.

There is a tendency for picnickers to develop immunity to signs just as flies develop immunity for DDT. And it doesn't matter whether the sign says "No Trespassing" or "Poisonous Shellfish". Nothing happened for a long time. However, if you could have visited Lepreau Basin at low tide on June 22 or 23, 1957, you could have witnessed the last scene of a little drama that everyone mistook for a farce. There you would have seen happy picnickers, mostly from Saint John, clustered around cooking fires along the margin of the beach. Some were roasting or shelling and eating clams. Others were reclining on the beach in the pleasant spring sunshine or in the shade of the red warning signs. The rest of the story you know. It was reported in last year's July 1 issue of this Journal.[4] No picnickers died, fortunately, but many became ill enough to be very, very interesting "cases".

For us, the important point is that the control system worked, in 1957, just as its design would call for—poison was detected by the Laboratory of Hygiene in extracts of shellfish prepared at the St. Andrews Fish Inspection Laboratory, and the Department of Fisheries, as it had agreed, put out bright new signs to warn the picnickers who swarmed down the beaches. But, although the system worked, it obviously did not work as it was intended to. The designers had made an important mistake (as so many of us make mistakes) in basic assumptions. They assumed that because picnickers would see the signs, they would read them. They also assumed that when picnickers read the signs they would follow the advice and not eat clams. People, of course, are often not that reasonable, and so we had our 1957 epidemic of paralytic shellfish poisoning.

I must emphasize that as far as control of the industrial harvesting was concerned, the system

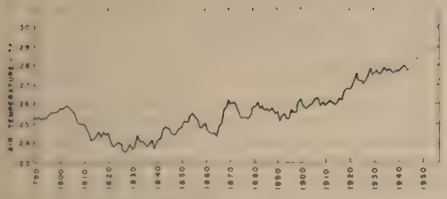

Fig. 2.—Glude's[4] figure showing trends in mean winter air temperatures in the northwest Atlantic region since 1790.

did work very well. The trouble stemmed almost entirely from picnickers.

CONTROL IMPROVED

In 1958 and 1959, there was a much better effort to protect picnickers in the Fundy area. This was instituted by Mr. R. M. Bond of the Fish Inspection Laboratory at St. Andrews. This responsibility had been assigned to him and warning signs were put out as before. But besides this, health officers were informed and newspapers and radio and television stations issued warnings as soon as the danger appeared imminent. All these kinds of warning work in different directions and they must continue as a necessary part of our control system. Still, we are not quite happy with our ghost—some of us, at least, are not.

HOW TO STRENGTHEN CONTROL

Since 1957 we have become aware of other needs besides that for information and education of the public. You begin to hear people saying that we should be in a position to make better forecasts and provide earlier warnings. There is a feeling that our system needs strengthening and now there is reason to believe that this need is increasing.

Let me show you what I mean. From Fig. 2, which comes from another author,[6] you will note that from about the year 1830 to 1955 our coastal climate was slowly warming. Hydrographers and climatologists assured us several years ago[7] that, beginning about 1955, this trend would be reversed and that a period of cooling would set in and would last approximately 25 years. What we have experienced since 1955 certainly bears out that prediction. The change is quite apparent in the Bay of Fundy. Yearly average water temperatures have been decreasing. Fig. 3 suggests how this change may affect the poison picture. I am not sure how much faith to put in this graph but if it has any meaning at all it is ominous. It indicates that in summers following cold winters the poison conditions are worse than in summers following mild winters. With what the hydrographers have predicted our graph carries this important warning—in the next 25 years we must expect not only colder winters but also worse trouble from shellfish poison. In the face of this you must agree that we should prepare ourselves to meet the trouble with the best forecast and control systems we can devise.

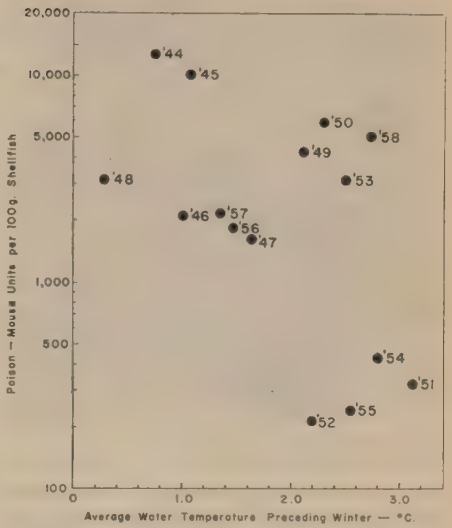

Fig. 3.—Relationship between annual means for poison content of all mussel samples taken at the Key Station (Head Harbour, N.B.) and means for water temperature (Biological Station wharf, St. Andrews, N.B.) for the three months of the preceding winter.

Besides being able to predict the danger period for each year we should try to find out how to forecast the level of the danger during that period. As we have seen, picnickers come to regard government warnings of danger as purely perfunctory and will sometimes completely disregard them. In case this disregard should become common in any particular year, it would be well to have some idea whether we should then expect to encounter only an occasional case of poisoning or a full-scale epidemic. If we knew that the danger in a particular year was likely to be great, the warning effort could be increased to ensure adequate control.

In the last few years we have found that our present method of forecasting sometimes provides a dangerously short warning period. With the climatic changes that are going on, we really should try to devise a longer-range method to provide more time, not only for the routine work of closing down commercial harvesting and posting dangerous areas with signs warning picnickers, but also for the recently adopted and more time-consuming types of warning through public health officers, newspapers, television and the like.

I think I have said enough about the parts of our control system which could be strengthened. Our system is good but no system is good enough if it can be made better at reasonable cost. So the next question is—how can we make it better?

KEY TO BETTER FORECASTING

The basis for our current forecast of when shellfish will become dangerously poisonous is the demonstration that they already contain sub-

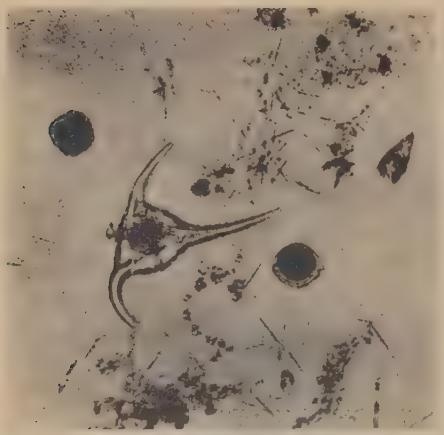

Fig. 4.—Photomicrograph of two specimens of the dinoflagellate, *Gonyaulax tamarensis* (actual diameter 40 μ), with other plankton organisms.

stantial and increasing amounts of poison. This demonstration is possible only at a late stage in a long chain of events that can end in trouble—a population of the causative organism must have built up in the sea, shellfish must have fed on it and accumulated poison digested from it, and collectors from the control agencies must have sampled the shellfish and submitted them to several time-consuming treatments before shipping extracts of them for assay to the Laboratory of Hygiene several hundred miles from the coast.

From reviewing Mrs. Needler's valuable work I feel almost confident that this elaborate system could be simplified. Here (Fig. 4) is the organism she considered to be basically responsible for all our trouble in the Fundy.[5] It is the dinoflagellate, *Gonyaulax tamarensis*. I do not know whether she was aware of it or not, but 12 years before she published her report Sommer[8] and his California co-workers suggested that this species might be responsible for poisonings in northern Europe. My belief is that we should re-open Mrs. Needler's investigation. If we could discover (1) the conditions which permit the development and maintenance of a population of this organism and (2) the conditions which determine the density of that population, once it establishes itself, then we would have everything we have been looking for as a basis for a long-range forecasting system.

I think there is a reasonable chance of discovering these. Furthermore, I think it would be cheaper, in the long run, to monitor these conditions and do the necessary forecasting than to maintain our present complex system that may break down sometime in the next 25 years when we need it most.

My guess is that the biology of dinoflagellate populations should be the first target of any research that aims to improve our control system. This is not a new idea by any means. The west coast Indians are said to have had a warning system[9] based on their own methods of estimating the abundance of dinoflagellates. They had no microscopes, but their system apparently worked. We are told that they watched the sea at night for luminescence. This is caused by dinoflagellates that often accompany *Gonyaulax catenella*, the source of shellfish poison in Pacific coast areas. When they observed a brilliant night glow in the sea they are said to have given up eating clams until they considered them safe again. They knew our ghost and when it walked.

Summary

The history of paralytic shellfish poisoning in Canada, especially in Bay of Fundy areas, and of research on the causative organism, *Gonyaulax tamarensis*, is reviewed. The Government's control of this public health hazard is outlined and it is predicted that the hazard will be greater in the next 25 years. A biological study of *Gonyaulax* might discover better methods of predicting annual danger periods and the level of toxicity of shellfish.

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THE EFFECT OF DIETARY MARINE FISH OILS ON THE SERUM CHOLESTEROL LEVELS IN HYPERCHOLESTEROLEMIC CHICKENS[1]

J. D. Wood and Jacob Biely[2]

Abstract

An investigation was carried out into the effect of marine fish oils on the serum cholesterol levels in hypercholesterolemic chickens. The oils were fed at the 10% level in the diet. Lingcod liver oil and halibut liver oil prevented the hypercholesterolemic effect of supplementary cholesterol, whereas crude herring oil increased the hypercholesterolemia to the same extent as did corn oil and tallow. Ratfish liver oil, dogfish liver oil, and basking shark liver oil had less noticeable effects on the cholesterol levels. No clear explanation could be given for the observed behavior but there seemed to be a difference in the effects on serum cholesterol of liver oils from teleostei fish and from selachii fish, the former class of liver oils being much more potent in preventing the increase in serum cholesterol concentration in chickens caused by the addition of cholesterol to the diet.

Introduction

The effect of dietary constituents on cholesterol levels in the blood of both humans and animals has been studied extensively in recent years. Among the constituents studied in greatest detail are the fats and oils. It has been found that unsaturated vegetable oils in the diet lower the blood cholesterol levels in humans (1, 2, 3). Similar effects have been observed on the blood cholesterol level of rats made hypercholesterolemic by the addition of cholesterol and cholic acid to the diet (4). However, unsaturated vegetable oils have the opposite effect when they are fed to chickens. These species differences are reviewed by Stamler (5).

The exact nature of the compound or compounds present in the vegetable oil which is responsible for the changes in the cholesterol levels is not yet known. Some workers believe the effect is due to the fatty acid components of the triglycerides, more especially the polyunsaturated acids (6). On the other hand, Beveridge and co-workers (7) believe that the active factor is contained in the unsaponifiable fraction of the oil. Hegsted *et al.* (8) have postulated that the polyunsaturated fatty acids responsible for the decrease in serum cholesterol values must belong to the "essential" fatty acid class. These workers claimed that "non-essential" polyunsaturated fatty acids, such as eleostearic acid, had the opposite effect.

The fatty acid composition of marine fish oils is quite different from that of animal fats or vegetable oils. The marine oils contain moderately large amounts of highly unsaturated fatty acids (9) but little or none of the "essential" fatty acids. The amount of the highly unsaturated fatty acids varies considerably in oils from different species of selachii fish, the higher the unsaponifiable content of the oil, the less the degree of unsaturation of the component fatty

[1]Manuscript received August 24, 1959.
Contribution from the Fisheries Research Board of Canada, Technological Station, University of British Columbia Campus, Vancouver, B.C.
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acids (9). The unsaponifiable fraction of marine oils varies greatly from one type of oil to another, not only quantitatively but also qualitatively (10, 11, 12, 13).

The effect of individual fish oils on cholesterol levels has been reported. Anderson *et al.* (1) found that sardine oil depressed the serum cholesterol level in man, and de Groot and Reed (14) showed that cod liver oil had a similar effect in rats. They found that the unsaponifiable portion of the cod liver oil did not contain the depressant factor.

In view of the facts outlined above, an investigation was carried out into the effects of various marine oils on the serum cholesterol levels in chickens. The oils were selected so that a wide variation in the composition of the oils was obtained with respect to fatty acid composition, degree of unsaturation, and amount of unsaponifiable material. It was thought that such an investigation would yield useful information into the part played by the components of the oils in the alteration of serum cholesterol levels.

Materials and Methods

Birds

One-week-old New Hampshire cockerels which had been fed the standard diet were used for the experiments. The birds were standardized by weight to avoid any possibility of variations in serum cholesterol levels due to differences in the size of the chickens.

Diets

The standard diet was composed of wheat 54.9%, yellow corn 10%, oats 5%, wheat middlings 5%, wheat bran 5%, soybean oil (44% protein) 7.5%, meat meal (50% protein) 5%, herring meal (70% protein) 2%, distillers' dried solubles 2.5%, dehydrated cereal grass 1.25%, iodized salt 0.5%, limestone 1%, manganese sulphate 0.015%, feeding oil (2250A-300D/g) 0.25%, nicarbazine 0.05%. Riboflavin was added in the proportions 0.05 g/100 lb feed.

Cholesterol and oils were added to the standard diet as shown in Table II in the concentrations of 1% and 10% by weight respectively.

The fish oils employed in the investigation, with the exception of the herring oil, had been filtered through Hyflo Super-Cel to remove traces of water and other contaminants. The tallow used as a dietary supplement was Sta-Y-fat, which is a stabilized animal fat for use as a feed supplement (guaranteed 20 AOM minimum).

Experimental Procedure

The chickens were divided into groups of 20 and each group was placed in a separate cage and fed the appropriate diet and water ad libitum. After 7 days on the diets the chickens were bled from the wing and 0.5 ml blood collected from each bird. The blood from four birds was pooled to give a sufficient quantity for the cholesterol determinations and the serum collected in the normal manner. There were therefore five pooled samples of serum from birds on each of the diets. The procedure was repeated after 13 days on the experimental diets. The amount of each diet consumed by the chickens was measured after 7 days and 13 days.

Examination of Cholesterol in Blood Serum

The determinations were carried out using the method of Zlatkis, Zak, and Boyle (15). This method, not being specific for cholesterol, was shown to have limitations (16). Preliminary experiments were run and serum cholesterol determined by the above method, and by the more specific method of Sperry and Webb (17), both on the serum from birds fed the standard diet and on the serum from chickens eating the diets with cholesterol, tallow, and oil supplements. Similar results were obtained by both methods of analysis except that the Zlatkis, Zak, and Boyle method gave values which were 10% higher. The latter method was chosen for the routine analysis of the serum cholesterol, because it was less time consuming.

Analysis of the Fat and Oils

The iodine absorption number of the compounds was estimated using the Hanus method as described in the *Official methods of analysis* of the A.O.A.C. The amount of unsaponifiable material was determined as described in the same handbook.

Results

Analyses were carried out on the fat and oils which were used to supplement the diets of the chickens (Table I). The amount of unsaponifiable matter in the

TABLE I

Composition of fat and oils used in the feeding experiments

Product	Iodine absorption number	Unsaponifiable material, %
Dogfish liver oil	114	14.3
Ratfish liver oil	90	26.1
Basking shark liver oil	203	42.9
Lingcod liver oil	143	3.7
Halibut liver oil	152	16.3
Crude herring oil	136	1.1
Corn oil	127	0.9
Tallow	63	0.5

fat and oils was extremely variable. In corn oil, tallow, crude herring oil, and lingcod liver oil it amounted to a few per cent of the whole at the most, whereas in halibut, ratfish, dogfish, and basking shark liver oils there were large amounts of unsaponifiable material. The unsaponifiable fractions were solid except that from basking shark liver oil which was liquid at room temperature. The major unsaponifiable component of this oil is squalene (13), a C_{30} hydrocarbon containing six double bonds. Ratfish liver oil and dogfish oil differ from the other oils used in this investigation in that their high unsaponifiable content is composed mainly of the glyceryl ethers, chimyl, batyl, and selachyl alcohols (10, 12). The first of these compounds is saturated and the third has one double bond in its structure.

The oils used to supplement the diets therefore varied considerably in composition. Some contained mainly triglycerides whereas others contained in addition large amounts of squalene or glyceryl ethers.

Table I also shows the iodine absorption numbers of the fat and oils. The oils chosen for this study exhibit a wide range of iodine values. The iodine absorption number in vegetable oils is often taken as a measure of the unsaturation of the component fatty acids because the oils consist mainly of triglycerides. The same does not apply to marine fish oils since the unsaponifiable material sometimes constitutes a significant portion of the oil and hence the degree of unsaturation of the unsaponifiable material strongly influences the iodine value of the oil. For instance, the high value observed here for basking shark liver oil is due to the high content of squalene (iodine value = 371). Similarly the low iodine values for dogfish and ratfish liver oils are due to their high content of the poorly unsaturated glyceryl ethers. The tallow, corn oil, and crude herring oil used in the investigation contained small amounts of unsaponifiable material and their iodine values give some indication of the amount of unsaturation in the component fatty acids. Tallow had the lowest degree of unsaturation followed by corn oil and herring oil in that order.

Table II gives the serum cholesterol levels of chickens which had been fed diets supplemented with cholesterol and various oils. The addition of 1%

TABLE II

The effect of dietary fat and oils on the serum cholesterol concentration in chickens

Addition to the standard diet	Food consumed, g/chicken		Serum cholesterol, mg/100 ml	
	7 days	13 days	7 days	13 days
None	97	193	185 ± 6	203 ± 27
1% cholesterol	95	191	314 ± 77	403 ± 51
1% cholesterol + 10% dogfish liver oil	68	156	252 ± 44	321 ± 119
1% cholesterol + 10% ratfish liver oil	75	179	217 ± 27*	262 ± 54*
1% cholesterol + 10% basking shark liver oil	79	190	313 ± 82	374 ± 96
1% cholesterol + 10% lingcod liver oil	57	117	192 ± 15**	155 ± 16**
1% cholesterol + 10% halibut liver oil	35	79	165 ± 12**	147 ± 21**
1% cholesterol + 10% crude herring oil	59	134	511 ± 65**	697 ± 161**
1% cholesterol + 10% corn oil	52	129	646 ± 140**	798 ± 219**
1% cholesterol + 10% tallow	52	136	432 ± 139**	662 ± 83**

*Analysis of variance indicates difference from "standard + 1% cholesterol" diet is significant at $P = .05$.
**Analysis of variance indicates difference from "standard + 1% cholesterol" diet is significant at $P = .01$.
NOTE: Each value in the table is the mean for five groups of serum ± the standard error.

cholesterol to the standard diet produced hypercholesterolemia in the chickens which was evident after both 7 days and 13 days on the diet. These hypercholesterolemic chickens were used as the controls for the diets supplemented with the fat and oils because the latter diets also contained 1% cholesterol. The effects of the dietary supplements were the same after 7 days on the diets as they were after 13 days on the diets. Analysis of variance showed that after both time intervals the diets containing lingcod liver oil and halibut liver oil significantly lowered the serum cholesterol concentration below that of the controls. The addition of tallow, corn oil, and crude herring oil to the diets significantly increased the serum cholesterol levels. Ratfish liver oil, dogfish liver oil, and basking shark liver oil did not bring about changes which were comparable to those effected by the other oils. The diet supplemented with ratfish liver oil produced a decrease in the cholesterol concentration which was

significant at the 5% level of significance but not at the 1% level. The other two oils caused no significant change. Tallow and corn oil were included in the experiment for comparative purposes. Both these products increased the cholesterol concentration in serum to about the same extent as crude herring oil.

The amount of food consumed by the chickens is shown in Table II. The addition of fat or oils to the diet reduced the amount of food consumed because of the high energy content of the diet.

Discussion

The results presented here indicate that dietary marine oils vary greatly in their effect on serum cholesterol levels in chickens. No relationship appears to exist between the quantity of unsaponifiable matter in the oils and the serum cholesterol concentration. Dogfish liver oil and halibut liver oil contain approximately equal amounts of unsaponifiable matter but the former has no effect on cholesterol levels whereas the latter reduces significantly the cholesterol concentration. Basking shark liver oil has a very high unsaponifiable content but the oil has no effect on serum cholesterol levels. It may be argued that this is due to the comparatively small amount of triglyceride consumed by the chickens since the amount of that component was, at the most, 57% of the total weight of the oil. However, when the food consumption is taken into consideration it is seen that the amount of lingcod liver oil consumed was 62% that of the basking shark liver oil. In other words, the amount of triglycerides consumed by the chickens on these two diets was approximately the same, yet one oil had no effect and the other a marked effect on the serum cholesterol levels.

No relationship appears to exists between the iodine absorption number of the oils and their effect on serum cholesterol levels. For example, lingcod liver oil and crude herring oil have similar iodine values but they have opposite effects on the serum cholesterol. Tallow has a much lower iodine absorption number than either corn oil or crude herring oil but all three oils cause similar increases in the serum cholesterol concentration. However, the interpretation of the data is complicated by the fact that the unsaponifiable material influences the iodine value of the oil as mentioned previously.

There may be a possible difference between classes of fish in the effect of their oils on cholesterol levels. The results presented here indicate that the teleostei or bony fishes (lingcod, halibut) significantly lower the cholesterol content, whereas selachii or cartilaginous fishes (ratfish, dogfish, basking shark) have a much less pronounced effect. The degree of unsaturation of the component highly unsaturated fatty acids in liver oils of selachii decreases as the amount of unsaponifiable material increases (9), the oils with low unsaponifiable contents having a degree of unsaturation similar to that of teleost liver oils. This may explain the difference in effects of the teleostei and selachii liver oils observed here, because the latter oils were all high in unsaponifiable material and therefore low in the amount of unsaturation of their component fatty acids.

The addition of herring oil to the diet led to an interesting effect. Although herring is a teleost fish the addition of its oil to the diet of the chickens increased

the serum cholesterol concentrations. However, this oil is not strictly comparable to the other teleost oils because, unlike the others, it was obtained from the whole fish rather than just the liver. In addition, the herring oil was crude and had not been subjected to the filtering operation which the other oils had undergone. A more thorough study of this topic will form the subject for a future investigation.

In summary it may be said that the results presented here indicate that there is no simple relationship between the amount or unsaturation of the component fatty acids in the oil and the serum cholesterol levels. However, the highly unsaturated fatty acids present in marine oils may play some part in reducing the cholesterol concentration. It would appear that "essential" fatty acids are not necessary for lowering cholesterol levels since fish oils contain little or none of this type of fatty acid. On the contrary, corn oil which contains large amounts of "essential" fatty acids produced the highest serum cholesterol levels observed during the investigation.

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ON THE ORIGIN OF THE CASPIAN AND BAIKAL SEALS AND THE PALEOCLIMATOLOGICAL IMPLICATION*

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ABSTRACT. The Caspian and Baikal seals, closely related to the ringed seal of the Arctic seas, are usually held to have arrived as a result of Pleistocene events. However, evidence suggests that they are relicts of late Miocene connections with the Arctic basin. The three species are more differentiated than is generally supposed. More important, some of the fossils from the upper Miocene of southern Russia and Hungary (and perhaps elsewhere) belong to the same subgenus (*Pusa*), and remains of the present Caspian seal are found in lower Pleistocene deposits, formed before the putative glacial entry of seals.

The fossils most readily diagnosed as belonging to the ringed seal group are an anterior fragment of a skull attributed to *Phoca pontica* by Alekseev and part of a lower jaw named *Praepusa pannonica* by Kretzoi. Some of the syntypes of *Phoca pontica* Eichwald (1853) also belong to this group and a lectotype is accordingly chosen here to prevent further confusion of the name. Much confusion has resulted from the identification by Simionescu as *Phoca pontica* of a form with a peculiar, distally expanded femur; this species is named *Pontophoca sarmatica* (Alekseev) n. comb. The synonymies of other forms which have been involved with the status of *Phoca pontica* are reviewed and lectotypes chosen when necessary.

The northern seals (subfamily Phocinae) are relatively cold stenothermic and probably entered the Sarmatian basin by direct connections to the north. The pups of living Phocinae have white coats as an adaptation to birth on ice, and this is almost certainly of common ancestry; the three species of the subgenus *Pusa*, within this subfamily, even more probably retain this adaptation from a common ancestor. Thus, if the ancestors of the Caspian seal were present in late Miocene times, ice almost certainly formed then, at least in the Arctic basin, and probably in the innermost reaches of the Sarmatian Sea.

INTRODUCTION

The presence in two great Asian lakes of seals closely related to the ringed seal of the Arctic seas has naturally excited much speculation. This note is prompted by certain Russian and eastern European papers which present views on the subject which are not widely recognized. Basically two theories have been put forward to explain the origin of these seals. The first presumes that they arrived as a result of Pleistocene events, and this is the only theory to be found in the zoogeographical literature of western Europe and America, as far as the author is aware. The second theory supposes that the Caspian seal and probably the Baikal seal are relicts of older, Tertiary connections with the northern seas. This paper attempts to show that the latter is the correct view.

The seals involved are members of the subfamily Phocinae of the hair seal group (family Phocidae). The systematics of the subfamily have been reviewed recently by Chapsky (1955a), whose views are substantially adopted by Scheffer (1958).

Many seals produce and nurture their pups on ice, but only the living members of the subfamily Phocinae (except for the specialized and probably early-separated bearded seal, *Erignathus*) are apparently derived from a single ice-breeding common ancestor. They are all related on anatomical grounds, of course, but more significantly their pups all have a white natal

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coat (lanugo). One, the harbor seal (*Phoca vitulina*) of the Atlantic and eastern Pacific, now breeds on land and the pup sheds the white coat *in utero*. The gray seal (*Halichoerus*) has turned to land-breeding in parts of its range, surely recently, for the pup retains the white coat. In all others the pup is produced on ice, where the functional value of the white coat is obvious in protective coloration and perhaps thermoregulation. It is improbable that this adaptation developed independently in seven species of the same subfamily. In the Antarctic, where predation (until man came) was not a factor, the white coat is not found in the five species of hair seal which may pup on ice. In the north Atlantic, the hooded seal (subfamily Cystophorinae) and the bearded seal (mentioned above) have failed to develop this feature. The importance of these considerations will become apparent.

The ringed, Caspian, and Baikal seals are members of the genus *Phoca*. Their subgenus, *Pusa*, has recently been raised to generic rank by Scheffer (1958), but reference to *Phoca* is preferred as it implies a relationship to the harbor seal group (*Phoca vitulina*) rather than to the other members of the subfamily Phocinae (the more specialized *Pagophilus*, *Histriophoca*, and *Halichoerus*). This follows the recommendation of Chapsky (1955a).

The Caspian seal (*Phoca caspica*) and the Baikal seal (*P. sibirica*) were described in 1788 by Gmelin, who thought them to be varieties of the harbor seal (*P. vitulina*). However, it became apparent that they were allied to the ringed seal (*P. hispida*), their affinities being discussed in detail by Nordqvist (1899), who relegated them to subspecific status. Seals have even recently (Mohr, 1952) been listed as possibly living in the Asian lakes Oron, Aral, and Kuku-nor, but this apparently is a mistake, for no seals are found in these lakes today (Ognev, 1935; apparently not noted by Mohr).

The possibility of Pleistocene origin will be discussed first. It will not be elaborated here in detail, since its refutation depends on confirmation of the opposing theory.

THE THEORY OF PLEISTOCENE ORIGIN

To account for the presence of Arctic elements, including the seal, a fish, and several Crustacea, in the Caspian Sea, Högbom (1917) assumed that a late glacial emigration from the Baltic region had occurred. The mechanism involved damming by glacial ice of northern lakes with the appropriate relict fauna, causing them to overflow into the Volga drainage and thence into the Caspian basin. Two of the relicts (the fish *Stenodus*, and the amphipod *Gammaracanthus*) are unknown from the Baltic or its relict lakes, and this led Pirozhnikov (1937) to propose that the northern fauna had been sent southwards from ice-dammed lakes formed by the west Siberian ice sheet.

Davies (1958a), who recently treated pinniped zoogeography as a whole, has accepted Pirozhnikov's theory and has attempted to elaborate and date the sequence of events. He supposes that during the third glaciation, which was the most extensive, the ice sheet formed first on the highlands of Novaya Zemlya and the Taimur Peninsula, spread southward to dam up the Ob and Yenesei Rivers, and picked up the seals from the gulfs of these rivers. The seals thus became inhabitants of the lake formed along the southern margin of

the Siberian ice sheet. There is geological evidence for a lake or lakes, which were controlled by a spillway at the head of the Tobol River and discharged into a route leading to the Aralo-Caspian basin (Flint, 1957). There is no evidence, however, for a lake as extensive as that which Davies has drawn (his figure 7, 1958a), incorporating the Caspian and Aral Seas and extending to the Central Siberian Highland. Lake Baikal drains into the Yenesei River, but is at present 300 meters above any possible ice-dammed lake. Davies cites evidence for considerable epeirogenetic uplift during the Pleistocene, and supposes that Lake Baikal would have been more accessible during the third glacial advance.

THE EVIDENCE FOR TERTIARY ORIGIN

The other opinion, that the Baikal and Caspian seals are relicts of pre-Quaternary connections of these waters with the northern seas, is not new. It was first put forward in the last century (by Andrussov, 1888; cited by Chapsky, 1955b), but only recently has the idea been given the full attention it merits by Chapsky, whose views form the basis for much of the argument presented here.

First of all, the degree of differentiation of the two lake-dwelling seals must be clarified. Nordqvist (1899) considered them to be subspecies, thus expressing their greater difference from the northern ringed seal than found in the "varieties" inhabiting the Baltic lakes Ladoga and Saima, which were obviously of recent, post-glacial origin. Subspecific status is still commonly accorded to them in the literature of northern Europe (Mohr, 1952; Eckman, 1953; Segerstråle, 1957) and this status would accord with a presumption of their Pleistocene origin. However, most recent authors concerned with their systematic position have given them specific status, if only because of their isolation.

The ringed seal of the Arctic and north-boreal seas is quite variable, and has been divided into a number of subspecies. Doubtless Scheffer (1958) is correct in presuming that most of these are invalid. At any rate, probably none can stand as defined, for the descriptions fail to account for differences imposed by age and sex (Chapsky, 1952) and for ecotypical differences in size related to ice conditions and latitude (McLaren, 1958). The differences between the ringed, Caspian and Baikal seals are not of this order at all and have been underemphasized. Chapsky (1955a; 1955b) redescribes each species thoroughly, but all their characteristics cannot be repeated here. There are consistent, age-independent differences in the skulls of the species. The pelt of the ringed seal throughout its wide range is "ringed" or marbled, that of the Baikal seal is spotted, while the skin of the Caspian seal is an unmarked gray. There are differences in parasites and feeding habits. There is a pronounced difference in the reproductive ecology of the Caspian seal, which breeds gregariously on ice floes, whereas the other two construct solitary pupping-lairs in the snow overlying land-fast ice.

The Caspian seal appears rather more differentiated from the ringed seal type, although the two lake seals have some features in common not found in the ringed seal. Taliev (cited by Brooks, 1950) found the Baikal seal serologi-

cally closer to the Arctic ringed seal than to the seals of Lake Ladoga or the Caspian Sea. However, this result cannot be significant of relationships, for there is no doubt that the Ladoga seal is merely a recently isolated and little differentiated ringed seal.

Taken as a whole, these differences imply an earlier separation than if we dismiss the inland seals as being mere subspecies of the ringed seal. Now an argument based on degree of differentiation alone would not suffice to overcome the argument for Pleistocene origin. The latter theory could claim that the populations were, at least on arrival, very small, and that this combined with the isolation in new surroundings would certainly result in more rapid evolution. However, the evidence for a pre-Quaternary origin of the Caspian seal, at least, rests on the fossil record, which must supersede any theory based on the Recent forms alone.

Pre-Pleistocene fossils.—The Sarmatian and Pontian basins of the late Miocene and early Pliocene[1] have provided a surprising number of fossils of marine mammals. Among the more important papers describing these fossils are those of Alekseev (1924a; 1924b), Kretzoi (1941), Macarovici and Oescu (1941), Macarovici (1942), and Simionescu (1926). Kellogg (1922) gives a very thorough review of all earlier finds, and Friant (1947) lists and discusses most species described to that date. Seals of the tropical-Antarctic group (subfamily Monachinae) and the northern group (subfamily Phocinae) are represented.

One of the first species described was *Phoca pontica* Eichwald (1853) from the Sarmatian of Bessarabia and from two localities on the Kerch Peninsula, both listed as Pontian (e.g., Kellogg, 1922), but apparently Sarmatian (Alekseev, 1924a). The name has become badly confused, for the original description was based on material of more than one species, and the name has since been applied to still other forms. Since the identification and relationships of *Phoca pontica* are important to the argument, it is necessary first to discuss misapplications and dubious changes of the name, and to divest it of fossils which are unrelated to Eichwald's type material. After this we may redefine the species from the original description by choosing a lectotype and then discussing its relationships. There are other problems of relationships and synonymy among the Sarmatian species, but these are beyond the scope of the present paper.

The fossils named *Phoca pontica* from Bessarabia were shown by Nordmann (1860) to be from a much larger species, which Nordmann renamed *Phoca maeotica*. There is complete agreement among the several authors on the identification of at least the femur (fig. 1e) of this large form, although it has been removed from the genus *Phoca* by some. Trouessart (1897) recognized that it belonged to the tropical-Antarctic group and renamed it *Monatherium maeoticum* (the spelling *Monotherium* has priority; see Kellogg.

[1] There is controversy over the correlations involved, but Russian workers (e.g., Neiman, 1957) believe that the Pontian stage and some of the top of the Sarmatian stage are to be considered as Pliocene. Some of the Sarmatian fossils have been listed as middle Miocene, but this cannot be maintained. It is sufficient for the argument and for wider correlation to suppose that the events described here took place near the Miocene-Pliocene transition. Reference has been made to the Lexique Stratigraphique International for correlation with western Europe.

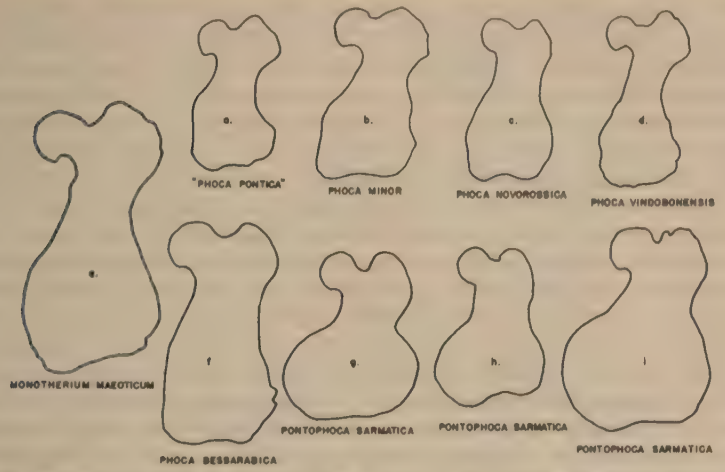

Fig. 1. Femurs from fossil seals. All to same scale (×1/3). Some reversed from originals for purposes of composition. (b and d from Friant, 1947; h from Simionescu, 1926; i from Macarovici and Oescu, 1941; others from type illustrations, as discussed in text.).

1922). The work of Nordmann should have resulted in the restriction of the name *Phoca pontica* to fossils from the small species of *Phoca* which formed important parts of the collection described, without designation of a holotype, by Eichwald.

Peters (1867) attributed seal fossils from the upper Miocene of the Vienna basin to *Phoca pontica*, without illustrating his material. Toula (1898) believed that these and more extensive remains were referable to a new species, *Phoca vindobonensis*.

Alekseev (1924a) described and figured from middle Sarmatian deposits of Kishinev the facial part of a skull and a fragment of a lower jaw, apparently from the same individual. These were from a small seal of the subfamily Phocinae and matched very well the small braincase from the Kerch Peninsula illustrated by Eichwald (1853) for *Phoca pontica*. Alekseev found skeletal material as well, which he neither described nor figured, but which he also referred to *Phoca pontica*.

Other bones, of which the femur seems to have been the source of most confusion, have been used to define the Sarmatian species. The femur (fig. 1a) considered to be part of the type of *Phoca pontica* by Eichwald was very small (65 mm. long) and resembles quite closely the femora of other species of *Phoca*, particularly *Phoca minor* (fig. 1b) from the Pliocene of Belgium, as illustrated by Friant (1947).

A grossly different form of femur (fig. 1h), with a very much expanded distal portion, was attributed to *Phoca pontica* by Simionescu (1926). Later Macarovici and Oescu (1941), following Simionescu, described a similar but larger femur (fig. 1i) under the same name. These are without doubt the same type of femur (fig. 1g) which had been defined earlier by Alekseev (1924b)

under the then new name *Phoca sarmatica*. Macarovici (1942), having newly discovered Alekseev's work, recognized this relationship and felt that the form described by Alekseev should be considered a variety, *Phoca pontica* var. *sarmatica*. Macarovici realized that these femora (fig. 1g, h, i) by no means matched the original illustration of the femur (fig. 1a) of *Phoca pontica*, but claimed that they fitted Eichwald's description of this bone. Actually, Eichwald's description agrees with his illustration quite adequately, and the measurements given in his text are exactly those of the illustrated femur. In the meanwhile Kretzoi (1941), who had not seen Alekseev's (1924b) work, recognized that the "*Phoca pontica*" of Simionescu (fig. 1h) had peculiarities which demanded its removal from the genus *Phoca*, and accordingly he renamed it *Pontophoca simionescui*. Friant (1947) had at hand an illustration of this peculiar femur which, following Simionescu, she believed to be representative of *Phoca pontica* Eichwald. Although apparently unaware of Kretzoi's work, she too recognized that this femur did not belong to the genus *Phoca*, and considered it to be allied to the modern tropical seal. Thus *Phoca pontica* Eichwald was termed *Monachus ponticus* (Eichwald) by Friant. In fact, this femur seems to bear little resemblance to that of the modern genus *Monachus*, or any other Recent genus. It is generically distinct and under the rules of priority it should retain the generic name given it by Kretzoi (1941) and the specific name given it earlier by Alekseev (1924b). It is therefore now designated *Pontophoca sarmatica* (Alekseev) McLaren n. comb. It is just possible that there are two types of seals involved under this name, in view of the wide range of size shown by the femora (fig. 1h, i), but the point to be made here is that they are not representative of, nor even related to the type material of *Phoca pontica*.

Macarovici (1942) also attempted to clear up confusion surrounding three other species of seals: *Phoca novorossica* Alekseev (1924b), *P. bessarabica* Simionescu (1926), and *P. vindobonensis* Toula (1898). In doing so, he reduced the second to synonymy with the first, on the basis of supposed resemblances of their femora and pelves. However, Alekseev stated emphatically that his *Phoca novorossica* was a small species, comparable in size with *Phoca vindobonensis* of the Vienna basin. *Phoca bessarabica*, as described by Simionescu and later by Macarovici and Oescu (1941), was a much larger form comparable in size with *Monotherium maeoticum*. The femora (fig. 1c, f) of the two forms that Macarovici considered synonymous actually bear little resemblance to one another and are respectively ca. 65 and 94 mm long. The pelves are more alike in appearance but show the same disparity of size. One might accept Macarovici's contention that size alone is not sufficient to separate these species, but at this point the status of *Phoca pontica* becomes involved. Since Macarovici had decided that the "real" *Phoca pontica* was represented by the peculiar femur discussed above (*Pontophoca sarmatica*), he concluded that the femur (fig. 1a) illustrated by Eichwald belonged to the species *Phoca novorossica* (fig. 1c), with which he considered *P. bessarabica* (fig. 1f) to be synonymous. There seems to be no justification for this identification of the femur originally described among the type material of *Phoca pontica*.

Kretzoi (1941) was unsatisfied with the supposed affinities of the femur and humerus illustrated by Eichwald as *Phoca pontica* and believed that they were referable to the Monachinae. He therefore proposed a new genus for the species and called it *Monachopsis pontica*, without designating a lectotype. No other worker seems to have considered this possibility, which appears unlikely as regards the femur at least, considering the small size of the bone and its resemblance to femora of other species of *Phoca*. The humerus is rather shorter and thicker than comparable bones from Recent *Phoca*, but these differences do not seem to be a sufficient basis for Kretzoi's new generic name.

Bogachov (1927) described a humerus from Sarmatian deposits near Baku on the Caspian Sea which was almost identical with that described by Eichwald among the type material of *Phoca pontica*.

Finally, Calvert and Neumayr (1880) have listed a few remains from Turkey which they believed were identifiable as *Phoca pontica*. Unfortunately, they gave neither measurements nor illustrations which would perhaps demonstrate the affinities of their finds, for at that time they seemed unaware of the possible existence of more than one species of seal in Sarmatian deposits.

There remains, then, the problem of redefining *Phoca pontica*, which has been cleared of some unrelated material, but which probably contained more than one species as described. For these reasons we must choose a lectotype from the type material of Eichwald (1853).

The fossils which were described in the original publication without designation of a holotype were all from the Kerch peninsula. Among them were a part of the cranium, a few teeth, some vertebrae, part of the sternum, a fragment of the scapula, the pelvic girdle, humerus, femur, radius, tibia, patella, some of the bones of the carpus and tarsus, and some phalanges. Eichwald mentioned the larger Bessarabian fossils, which later became *Monotherium maeoticum* (Nordmann, 1860), but these did not acutally enter into his description of *Phoca pontica*. Indeed, Eichwald thought that his fossil seal must have resembled closely the modern harbor seal, *Phoca vitulina*, and apparently used the skull of this living species as a model from which to outline the missing portions of his fossil skull. Thus we may take Eichwald's *Phoca pontica* as based on specimens from the small species of *Phoca* which were actually included in the collection which he described. Of those who have sought to define or rename *Phoca pontica*, Alekseev (1924a) was the first, and he alone has described further material (the anterior fragment of a skull) which has given us a more precise conception of this small species of seal and which agrees with at least part of Eichwald's type material. It is felt that the posterior part of the skull, well matched by the anterior part discovered by Alekseev (as shown here in fig. 2), should serve as the restricted type material, and it is therefore designated as the lectotype of *Phoca pontica* Eichwald. Other parts of the skeleton included in the original description are problematical. They were collected over a period of time from beds in two localities and we are given no information on which bones were found grouped together. For reasons which will be given later (p. 59) the first metatarsus illustrated by Eichwald can safely be placed in the same species as the skull. Although the femur appears perfectly suitable as part of a small species of *Phoca*, it has been the subject of so much controversy that it had best be excluded from

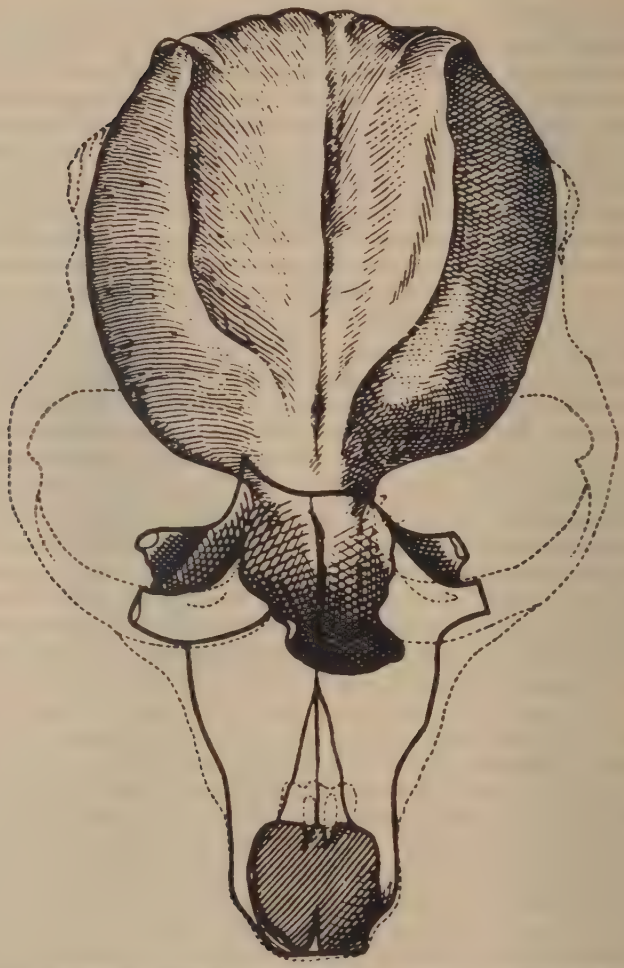

Fig. 2. The anterior fragment of the skull described by Alekseev matched with the posterior fragment illustrated by Eichwald (1853) as the type of *Phoca pontica* (×1). The dotted outline represents Eichwald's attempt to reconstruct the skull, using *Phoca vitulina* as a model. From Alekseev (1924a).

Phoca pontica for the present; the same is true of the humerus. Other bones should be referred to *Phoca pontica* only if they can be shown in future studies to belong to the same species of seal represented by the skull and first metatarsus.

Eichwald had supposed that the fragment of the cranium of *Phoca pontica* indicated close affinities with *Phoca vitulina*. In fact, the small size of the cranium which he has reconstructed, which is evidently from an adult, makes a relationship with the subgenus *Pusa*, or ringed seal group, far more likely.

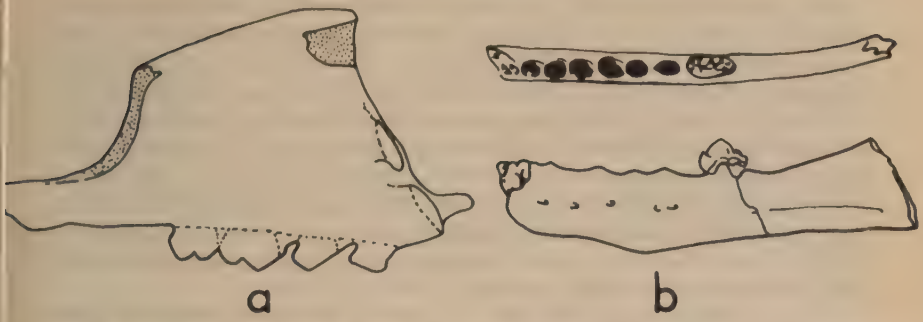

Fig. 3. Significant fossils from the upper Miocene (×1). At left (a) outline sketch from a photograph of the anterior fragment of the skull attributed to *Phoca pontica* by Alekseev (1924a). The teeth appear to be obscured by matrix in his photograph. At right (b) the lower jaw described by Kretzoi (1941) as *Praepusa pannonica*, as redrawn from his sketch.

The facial portion of the skull (outlined here in fig. 3a) which Alekseev (1924a) referred to *Phoca pontica* is most important. Alekseev compared it with skulls of *Phoca hispida*, *P. vitulina*, and *Pagophilus groenlandicus*, but did not ascribe it to any of these species. Although he found most points in

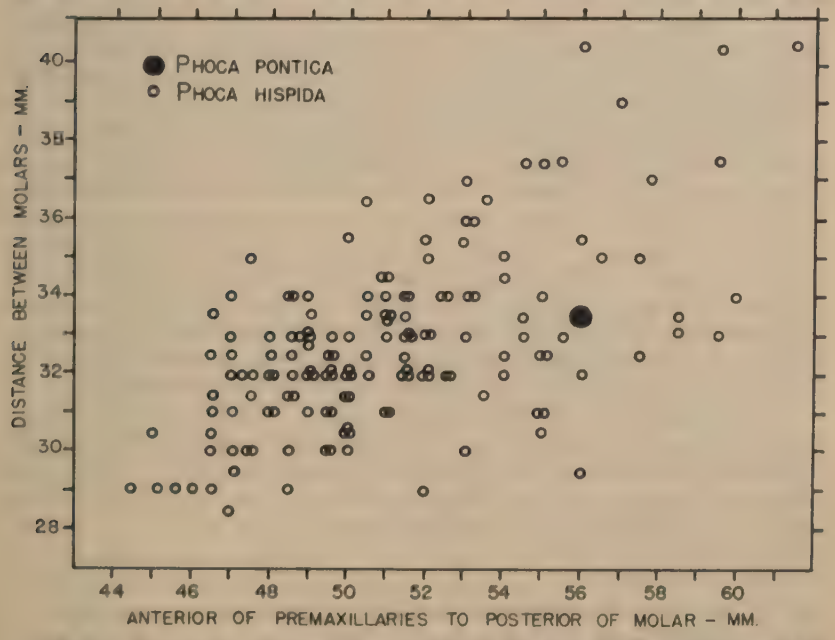

Fig. 4. Comparison of general form and size of the snout of *Phoca pontica* with the range shown by adult *Phoca hispida* (all aged at more than 7 years) from various localities in the Canadian Arctic. The distance between the molars (M_1) of *Phoca pontica* is listed by Alekseev (1924a); the distance from the anterior tip of the premaxillaries to the posterior margin of the molar was measured from his photograph.

common with *Phoca hispida*, he supposed that the muzzle was in general too elongate to resemble that of the living form. In fact, such elongation is typical of adult *Phoca hispida*. It is plain from figure 4 that Alekseev's specimen, here referred to *Phoca pontica*, does not differ from *Phoca hispida* in size or relative length of snout. A few years after Alekseev's study, Bogachov (1927) compared Alekseev's specimen with the equivalent part of the present Caspian seal and found many points in common. However, perhaps because he was misled by the skeletal material discussed above, it seemed impossible to Bogachov that *Phoca pontica* could be ancestral to the living form. Chapsky (1955b) has left no doubt that Alekseev's find belongs to the subgenus *Pusa* and the present author agrees completely with this conclusion.

The northern seals (Phocinae) can most readily be separated on the structure of the jaws and teeth, and Chapsky seemed unaware that Kretzoi (1941) had made a very significant find. Kretzoi described from middle Sarmatian deposits near Budapest a fragment of a mandible (fig. 3b) which is unquestionably of the subgenus *Pusa*, in view of its light structure and small last molar (the only tooth remaining). He was fully aware of its relationships and named it *Praepusa pannonica*. Since *Praepusa* has no characters separating it from *Phoca* (*Pusa*), Kretzoi's generic name must be changed to *Phoca*.

Bogachov (1927) believed that a humerus and a femur from Sarmatian deposits of the Caspian basin were referable to *Phoca vindobonensis* Toula (1898). These fossils were also noted as being very similar to the equivalent bones of the present Caspian seal, and Bogachov offered this as evidence of the late Miocene origin of the modern species. Chapsky (1955b) also stressed the resemblances between parts of *Phoca vindobonensis* and *P. caspica*.

Undoubtedly more of the bones of Phocinae described from Sarmatian deposits will prove to belong to the subgenus *Pusa*. In the author's opinion these fossils are best considered as marking a southward extension in the range of ancestors of *Phoca hispida*, which have since been isolated to give rise to the present Caspian seal. It seems unlikely that more than one species of this subgenus existed in Sarmatian waters, and while a conservative attitude must be taken at present, it is probable that fossils from this subgenus will be shown in future to be referable to *Phoca pontica*, as here restricted. Information is badly needed on the extent of variation of the limb bones of the living species in order that species may be more sensibly defined among the fossil seals.

The formal synonymy of the relevant species is summarized here. Lectotypes are chosen for all species in which a holotype was not designated and comments are offered on some of the referred material. Kellogg (1922) is the most useful source of further references, not involving the status of *Phoca pontica*, but concerning some of the listed species.

Subfamily Monachinae

Monotherium maeoticum (Nordmann)

Phoca pontica (p.p.) Eichwald, 1853.
Ph(*oca*) *maeotica* Nordmann, 1860.
Phoca maeotica Alekseev, 1924b; Simionescu, 1926; Macarovici and Oescu, 1941; Macarovici, 1942.

(*Monatherium*) *maeoticum* Trouessart, 1897.
Monotherium maeoticum Kellogg, 1922.
Monatherium maeoticum Friant, 1947.

Lectotype: The femur described and illustrated by Nordmann (1860, text p. 321, atlas pl. 23, figs. 8 and 9) as part of the type material.

Comments on referred material: According to Alekseev (1924b) the femur, humerus, radius, and pelvis in Nordmann's figures are attributable to this species, while the cubitus and the tibia belong to *Pontophoca sarmatica*.

Range: Lectotype from Sarmatian limestone in vicinity of Kishinev. Apparently confined to Bessarabian deposits.

Subfamily Monachinae?

Pontophoca sarmatica (Alekseev), n. comb.

Phoca sarmatica Alekseev, 1924b.
Phoca pontica Simionescu, 1926; Macarovici and Oescu, 1941; Macarovici, 1942.
Phoca pontica var. *sarmatica* Macarovici, 1942.
Pontophoca simionescui Kretzoi, 1941.
Monachus ponticus Friant, 1947.

Lectotype: The femur described and figured by Alekseev (1924b, p. 203, fig. 6) as part of the type material.

Comments on referred material: The humerus described and figured in the type material may belong with *Phoca bessarabica* according to Macarovici (1942). Other material acceptable as this species includes the femurs described as *Phoca pontica* by Simionescu (1926) and Macarovici and Oescu (1941).

Range: Precise locality of lectotype not given. Found in middle Sarmatian beds near Kishinev and also near Tsmiliansk on the Don River.

Subfamily Phocinae

Phoca (Pusa) pontica Eichwald

Phoca pontica (p.p.) Eichwald, 1853.
Phoca pontica Alekseev, 1924a; Kirpichnikov, 1953.
Phoca pontica (?) Bogachov, 1927. (See below).
Monachopsis pontica (?) Kretzoi, 1941. (See below).
Phoca novorossica (p.p) (?) Macarovici, 1942. (See below).
Not: *Phoca pontica* Peters, 1867.
Phoca pontica Simionescu, 1926; Macarovici and Oescu, 1941; Macarovici, 1942.
Phoca pontica var. *sarmatica* Macarovici, 1942.

Lectotype: The fragment of the cranium described and figured by Eichwald (1853, text p. 391; atlas pl. 13, fig. 1).

Comments on referred material: The metatarsus I of Eichwald's type material and the anterior fragment of the skull described by Alekseev (1924a) are safely referable to this species. Possibly also the femur illustrated by Eichwald, renamed *Monachopsis pontica* by Kretzoi (1941), and attributed to *Phoca novorossica* by Macarovici (1942). The humerus (also renamed as part

of *Monachopsis pontica* by Kretzoi and found in Caspian basin deposits by Bogachov, 1927) is problematical.

Range: Lectotype from upper (?) Sarmatian of Kerch Peninsula (Crimea), but precise locality not given. Other specimens were collected later from Sarmatian limestone near Kishinev and (possibly) from the Sarmatian of the Caspian basin near Baku.

The following are maintained as separate species, with the understanding that parts of any of them may in future be referable to *Phoca pontica* Eichwald or that synonymies may exist between them.

Phoca vindobonensis Toula

Phoca pontica Peters, 1867.
Phoca vindobonensis Toula, 1898, and all subsequent authors.
Phoca vindobonensis Bogachov, 1927.

Lectotype: The femur described and illustrated by Toula (1898, p. 62-63, pl. 2, fig. 2a, b, c) as part of the type material.

Comments on referred material: No problems of the affinities of the type material have been raised.

Range: Lectotype from Sarmatian near Vienna (Kreindl's brickyard on the Nussdorf road near Heiligenstadt). Since from Vienna basin and Sarmatian deposits near Baku, on the Caspian Sea.

Phoca novorossica Alekseev

Phoca novorossica Alekseev, 1924b; Friant, 1947.
Phoca novorossica (exclusive of parts belonging to *P. bessarabica*) Macarovici, 1942.
Not: Femur illustrated by Eichwald as *Phoca pontica* and referred to *Phoca novorossica* by Macarovici, 1942.

Lectotype: The femur described and illustrated by Alekseev (1924b, p. 203, fig. 10) as part of the type material.

Comments on referred material: Some parts of the type material may belong with other species according to Macarovici (1942). Although Macarovici thought the femur resembled that of *P. bessarabica*, this is not so (see fig. 1c and f here), and the femur is safely chosen as the lectotype. The small pelvis seems validly included with the femur of this species.

Range: Locality of lectotype not given. Found in Sarmatian of Bessarabia, Odessa, on the lower Dnieper River, and near Stavropol.

Phoca bessarabica Simionescu

Phoca bessarabica Simionescu, 1926; Macarovici and Oescu, 1941.
Phoca novorossica (p.p.) Macarovici, 1942.

Lectotype: The pelvis described and figured by Simionescu (1926, p. 187, pl. 2, fig. 1) as part of the type material.

Comments on referred material: Unfortunately, the femur had not been discovered when this species was described and the pelvis must serve as the lectotype. The femur was described and figured by Macarovici and Oescu (1941). Macarovici (1942) then reduced this species to synonymy with *P.*

bessarabica, but this is rejected here for reasons given above.

Range: Lectotype from middle Sarmatian limestone near Kishinev, and since only from that locality.

Phoca (Pusa) pannonica (Kretzoi)

Praepusa pannonica Kretzoi, 1941.

Holotype: As defined by Kretzoi (1941, p. 275, fig. 1), the fragment of a left lower jaw.

Comments on referred material: Kretzoi defined as paratypes the proximal and distal ends of the left tibia and the distal end of the left fibula.

Range: Type and paratypes from the lower middle Sarmatian (Ukrainium) of Erd, near Budapest (6 m. deep in Cerithian chalk on the property of Franz Bitter).

Pleistocene fossils.—Another form of paleontological evidence is the demonstration that seals very like the modern Caspian species were present during early Quaternary times. A fossil tibia, indistinguishable from the comparable bone of the present Caspian seal, has been described by Vershchagin and Gromov (1952) from a site on the Ural River, over 500 km. north of the shore of the present Caspian Sea. They could not give the precise stratigraphic position, but attempted to correlate it by indirect means with the Kvalinsk transgression of the Caspian Sea (last or Würm glaciation). This find cannot be adduced as evidence for a pre-Pleistocene arrival, for the proponents of Pleistocene origin suggest that the previous glaciation (third, or Riss) was responsible for the arrival of seals. It should be noted, however, that the Pleistocene fossils of ringed seals are in their turn indistinguishable from the modern species, *Phoca hispida* (see Kellogg, 1922).

A more significant fossil was described by Kirpichnikov (1953) from the south coast of the Sea of Azov, from deposits which are correlated with the Bakinsk transgression of the Caspian Sea of the early Pleistocene (second or Mindel glaciation). At this time the Black (Azov) and Caspian Sea basins were connected. The bone, a first metatarsal, was found in fluvial deposits, along with the remains of early Pleistocene terrestrial mammals (*Elephas meridionalis*, *Equus sussenbornensis*, etc.). Since the deposit contained redistributed fragments of the middle Sarmatian marine mollusc, *Cryptomactra pesanseris*, it had been considered possible that the seal bone was of Sarmatian age as well. However, Kirpichnikov rejects this conclusion by comparing the metatarsal with equivalent bones from one contemporary *Phoca caspica* and three upper Sarmatian seals referred to *Phoca pontica* (and wholly comparable with the first metatarsal illustrated by Eichwald, 1853). Indeed his measurements and figures do suggest that the fossil from the Sea of Azov matches best with *Phoca caspica*, but they also stress that there is very little difference between the metatarsal of the modern seal and those of the Sarmatian species. Kirpichnikov cites records of the entry of the present Caspian seal far into rivers as pointing to the way in which the fossil bone came to be found in fluvial deposits, along with remains of terrestrial mammals.

The evidence suggests, then, that a seal very like the modern Caspian seal was present in the Black Sea basin during early Quaternary times, before the

putative Riss glacial entry of seals into the Caspian Sea. Kirpichnikov does not concern himself with the possibility of genetic ties between the late Miocene seals and the modern form, but if his argument on the age of his fossil is disputed, the only alternative is that a seal very like the modern Caspian seal was present in the Black Sea basin during Sarmatian times, which has already been shown on other evidence.

Apparently other remains of seals (cited by Bogachov, 1927, Kirpichnikov, 1953, and Chapsky, 1955b) were found in the Bakinsk (lower Pleistocene) deposits of the Caspian Sea itself, but unfortunately these do not appear to have been compared with material from the Recent species.

If seals of the subgenus *Pusa* were thus present in Sarmatian waters, and if seals very like the present *Phoca* (*Pusa*) *caspica* were found there during early Quaternary times, then we must accept these as substantial refutations of the theory of later Pleistocene origin of the Caspian seal.

While there is no fossil evidence for the Baikal seal, it would be an economy of theory to explain their presence in the same way. We must finally fall back on the fact that the Baikal seal is more than slightly differentiated from the ringed seal.

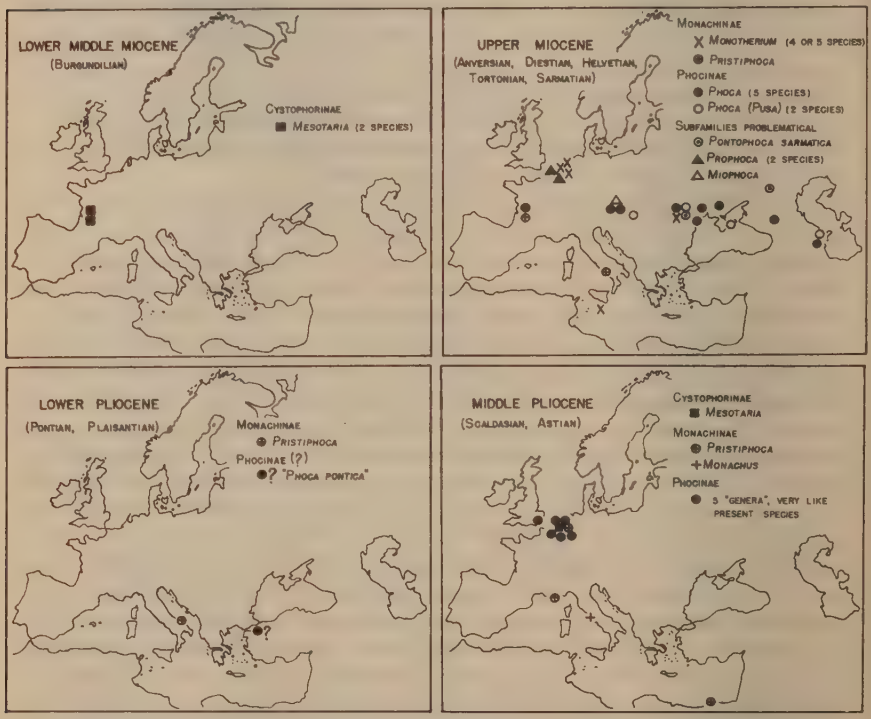

Fig. 5. Geographic and stratigraphic distribution of fossil seals in Europe. Each symbol represents a described species in each locality.

THE ROUTES OF ENTRY OF THE SEALS

It will be profitable to refer briefly to the overall fossil record (fig. 5). Although Cetacea are known earlier, the first fossils of seals in Europe are teeth found in the middle Miocene of southwest France. These are problematical, but are referred to the subfamily Cystophorinae (elephant and hooded seals).

Numbers of seals are described from the marine deposits of the upper Miocene, including those dealt with above. It can be noticed on figure 5 that, while the Sarmatian basin had several species of *Phoca* as well as seals of the tropical-Antarctic group, the Mediterranean and western European fossils are mostly of the Monachinae, except for a single femur from southwest France described as *Phoca couffoni* by Friant (1947) and two large hair seals (*Prophoca*), certainly not closely related to the modern northern seal group, from Anversian deposits.

The lower Pliocene has not produced much fossil material, but fragmentary remains from Turkey were referred to "*Phoca pontica* (see p. 53) and a seal (*Pristiphoca*) from Italy is close to the modern tropical seal (Friant, 1947, has in fact referred *Pristiphoca* to the genus *Monachus*).

In the middle Pliocene a whole host of species appears in the deposits of Belgium and Holland. One is of the Cystophorinae and one of the tropical-Antarctic group, while the others are "genera" (Friant, 1947, has reduced all but one to *Phoca*) quite close to the modern Phocinae. In the Mediterranean, the modern tropical seal was present.

Davies (1958b) has argued cogently for the origin of the Pinnipedia in the Arctic basin, and for the continuous restriction of the Phocinae to the northern hemisphere, by stressing the relative stenothermy of all except the Monachinae (all others live only in waters of less than 20°C maximum). There is little doubt that for the northern seal group during the late Miocene, the route around western Europe, through the Mediterranean, and into the Sarmatian Sea would have been too warm. The discovery of only one species of *Phoca* in deposits of that time in western Europe also suggests this (and *P. couffoni*, based on only one specimen, might bear re-examination). We must then consider a route of entry from the north.

Kretzoi (1941) saw no difficulty in connecting his *Praepusa pannonica* of Hungary with living seals of the same group, and with a zoogeographical flourish he created a "paratethys" in which the ancestral seals were developed. Along with two openings to the Arctic, one to the Mediterranean, and one to the Pacific, this Paratethys Sea was extended to cover Lake Oron, the Aral Sea, and Lake Kuku-nor, where no seals exist (p. 48). Kretzoi's Paratethys is purely imaginary, although his fossil find cannot be denied.

The northern shore of the Sarmatian Sea is difficult to restore with precision, since erosion has destroyed the deposits (Chapsky, 1955b). Although a connection between the Tethys Sea and the Arctic Ocean was present during early Tertiary, its existence during Miocene times seems problematical (Ekman, 1953). Nevertheless, it is felt that some connection must be invoked to get northern seals into southeastern Europe in the late Miocene. Seals are quite capable of moving into fresh water, and the connection need not have

been strictly marine. However, as well as the ringed seal group, other types of northern seals appear to have been present, including forms which may have resembled more the modern *Phoca vitulina* and *Halichoerus* (the jaw of *Miophoca vestuta* Zapfe is supposed by Chapsky, 1955a, to resemble that of *Halichoerus*). All in all, a fully marine connection seems more likely, although not absolutely necessary to the argument.

Probably the late Miocene marine transgressions, well known in Europe, extended either from the Arctic or from the Sarmatian Sea and brought the seals close to Lake Baikal. Certainly the fauna of this ancient lake, except for the seal and possibly an amphipod, has otherwise been isolated from marine invaders from a time earlier than late Miocene (Brooks, 1950). The Arctic ringed seal probably swims in and out of Nettilling Lake, Baffin Island, (see Manning, 1943) and the same capacity might have enabled the late Miocene seal to enter Lake Baikal, even if that lake were above sea level. Davies (1958) cites evidence for epeirogenetic uplift during Pleistocene, arguing that the entrance of the lake by the seal occurred during Pleistocene before that uplift; the same evidence might indicate that the lake was even more accessible in late Miocene.

In summary, the progenitors of the Caspian seal and probably of the Baikal seal can be considered to have arrived from the northern seas by connections, which were essentially marine, in late Miocene times.

THE IMPLICATIONS FOR PLEISTOCENE ZOOGEOGRAPHY

It can be seen that the demonstration of the probable Tertiary origin of the Caspian seal casts at least some doubt on the significance of the great Siberian ice-dammed lake as an agency in zoogeography. Certain Russian and western European authors have made wide use of this lake in their theories. Segerstråle (1957) used it to get an amphipod (*Pallasea*) into Europe during the third glaciation. All the northern elements in the Caspian Sea are explained by it (Pirozhnikov, 1937). Gur'ianova (1939) demanded that it be brackish for some zoogeographical purposes, and suggested that it has been concerned in establishing certain brackish water animals in North America as well. Perhaps a note of caution should be offered. Many of the relict species in lakes which have been analysed in the light of Pleistocene events are widespread (some Holarctic) and very successful. They are also geographically little differentiated, but this may reflect stability rather than recent isolation. It would be profitable to reexamine some of these species to determine whether they were established as fresh-water relicts before Pleistocene times in parts of their present range. This applies particularly to the presumed Pleistocene relicts of the Caspian Sea.

THE IMPLICATIONS FOR PALEOCLIMATOLOGY

As Chapsky (1955b) has pointed out, the presence of the progenitors of the Caspian seal in Sarmatian Seas implies that ice must have formed at that time. Actually, accepting the fossil evidence and its correlations does not absolutely necessitate that we believe ice formed in the Sarmatian Sea, but makes it almost certain that ice formed somewhere during that time, as the following arguments will show.

It might be argued, against all probability (see p. 48), that the white-coat pup arose independently in the several groups of the subfamily Phocinae. But the argument that it arose independently in the three closely related species of a subgenus within that subfamily is even more untenable. If one of this primitively ice-breeding group has lived continuously in the Caspian basin since late Miocene, then ice was almost certainly present somewhere at that time.

Although it seems more simple to suppose that the seals retained the ice-breeding habit in the Sarmatian Sea, it could be argued that they lost this habit when they spread into southern regions, where ice was not available. This has happened to different degrees in two modern species (see p. 48). The Caspian seal has lost the specialized relationship to fast ice shown by the ringed and Baikal species (see p. 49) and this might be taken as an indication of adaptation to periods when there was much less ice and snow than found today. It is also possible that the fossils of *Phoca pontica* from southern Russia are from seals which died far to the south of their breeding range. The modern Caspian species finds ice only in the north, although it migrates annually to the southern end of the sea.

In considering the climatological significance of the Monachinae, related to the modern tropical seals, it should be remembered that there were several types of Monachinae present, and that the modern group also includes four Antarctic species. The subfamily was quite likely Tethyan in origin, and their eurythermy may have permitted them to transgress the tropics during Miocene times, thence to colonize Antarctica (Davies, 1958b). Thus the admixture of fossils from the tropical-Antarctic and northern seals is not as paradoxical as it seems at first. Perhaps significant also are the facts that only near Kishinev is *Phoca pontica* found in the same locality with other seals, and *Phoca pannonica* is found alone. Unfortunately, the precise stratigraphic positions of many of the fossils are not given, but it is quite possible that seals of the subgenus *Pusa* were not strictly contemporaneous with seals belonging to the Monachinae.

There is also the problem of why only the Caspian seal of the several Phocinae present and none of the Monachinae should have survived to the present day. This can be attributed to post-Miocene climatic vicissitudes, and it is noteworthy that only the members of the ringed seal group normally keep open holes in the ice during an entire winter season, although other northern species may maintain holes briefly.

Other evidence can be brought forward to suggest that the climate of the late Miocene was sufficiently cool to form ice.

In the seas, there is evidence (in displacement of large boulders; Chapsky, 1955b) that ice was present in the Pontian Sea, at least. Ekman (1953) discussed the climatic deterioration in the Caribbean and elsewhere during Miocene. Today's marine temperatures were essentially reached in the Pliocene (the late Miocene was only a little warmer) on the west coast of North America (Durham, 1950).

MacGinitie (1958) has recently reviewed our knowledge of climate since the late Cretaceous, with special reference to the history of the floras of west-

ern North America. The late Miocene was certainly warmer than at present, although most of the southward migration of the forests was completed by that time. Climatic zonation certainly existed in late Miocene, and while the western United States seemed little influenced by polar air masses, MacGinitie suggests that winter conditions occurred and snow probably fell in the northwestern United States. The European floras seem to have been affected more by Pliocene cooling.

In conclusion, ice undobutedly formed in the northern seas and probably in at least the innermost reaches of the Sarmatian Sea during late Miocene. This emphasizes the statement of Barghoorn (1953) that

> the great trend of Cenozoic climate which culminated in Pleistocene glaciation began in the mid-Tertiary, probably more than 20 million years ago. Pleistocene glaciation itself . . . may then be regarded as a geologic and climatologic event the antecedents of which extend over a fair segment of recent geologic time and is not to be viewed as a sudden change in the history of the earth's climate.

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CANADA INSPECTED

GUIDE TO STANDARDS

PROCESSED UNDER GOVERNMENT SUPERVISION

PART 6

Handling, Chilling and Freezing Fish

by C. H. CASTELL[1], J. S. M. HARRISON[2] and O. C. YOUNG[3]

This article of the series deals directly with in-plant techniques which are designed to keep first quality fish free from contamination and spoilage throughout the entire processing stage.

Mr. Castell is the author of the section on the handling of fish; Mr. Harrison contributed that dealing with chilling; and Mr. Young completes the article with a section on freezing.

THE FOLLOWING DISCUSSION deals with fish from the time it has been unloaded from the vessels until it has been processed and ready for freezing or shipment as iced, unfrozen fillets. It will be taken for granted that we start out with good quality fresh fish from the vessel. During this period, while the fish is being stored and processed in the plant, two things can happen that may prevent the finished product from being acceptable according to the Canadian Government Specifications Board (C.G.S.B.) standards. First, there can be a decrease in the quality of the fish. This may occur to the whole or gutted fish prior to cutting; and it may also occur during the handling and storage of the fillets. The second problem is concerned with the number of bacteria on the finished fillets.

Although these two problems, the quality of the fish and the bacterial content, appear to be somewhat related, they are actually quite different. The first is concerned with the quality or *freshness* of the fish. When loss of quality occurs, it usually means that the round fish have been held too long or at too high a temperature before being cut; or the fillets may have been mistreated or improperly chilled after being processed. The second problem is chiefly a matter of plant sanitation and care in handling the fillets. A high bacteria count on the fillets indicates unsanitary surfaces or careless handling of the fish as they pass down the cutting lines. These two problems and their relation to C.G.S.B. specifications for handling and processing fish will be discussed separately.

Loss of Quality While Fish Are In The Plant

Loss of quality *in the plant* is not a frequent occurrence. When it does occur it happens mainly during the warm summer weather; and most of the in-plant spoilage is confined to the storage period before the fish are cut. At this period there are three main types of spoilage: the development of bilgy odours; the development of sour odours and general deterioration; and the development of soft-textured fish that are difficult to skin and easily flake apart.

Bilge-like odours may develop in fish that are stored in direct contact with dirty wood surfaces or slimy, unclean concrete floors. Hence arises the clause (5-4-2) in the specification, requiring that the bins or boxes used for storing fish in the plant must have smooth clean surfaces and be kept in a sanitary condition. Where the temperature is relatively high, this bilgy condition can develop on fish in the plant after a few hours in storage. Somewhat similar, very unpleasant, off-odours may develop where pockets of fish juice and slime are allowed to form near the bottom of tightly packed and improperly drained bins or boxes of fish. A layer of ice between the fish and the floor or the bottom of the container, prevents the development of these very unpleasant odours by preventing contact with the dirty surfaces and permitting proper drainage from the fish. A general souring and development of other off-odours occurs when fish are held too long without ice or when the ice is insufficient to properly chill them. It must be remembered that at the time they are discharged, many of the fish have already spent the best part of a week in the holds of the vessels. For this reason it takes a relatively short period of high temperature for many of these fish to develop off-odours. Clause 5-4-1 states that all fish not being processed immediately shall be adequately iced or chilled for holding. This is particularly essential during the warmer seasons of the year.

Temperature Affects Texture

In addition to this ordinary bacterial spoilage, some fish, particularly those caught in the summer, are very subject to softening. What otherwise appear to be good quality fish may become soft and even mushy if their temperature is allowed to increase to about 60°F. for a short time.

Where the fish are transported to

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the cutting tables by means of water-flumes, care must be taken to see that an increase in water temperature does not result in soft-textured fish. In most cases where the water is obtained directly from the sea the temperature will usually below 55° or 60°F., which is satisfactory. But, occasionally in the summertime, municipal supplies or water drawn from shallow lakes or rivers may increase to 60° or even 70°F. and higher. This frequently results in the production of soft fillets that are difficult to skin and which may flake apart during subsequent handling.

There is no serious difficulty in preventing the development of in-plant spoilage in the whole fish. The obvious methods are to reduce the storage periods to a minimum and to keep the fish well iced in clean, well drained bins or boxes or otherwise, chilled to 32°F. or lower. Although it is not always possible to control the temperature of the water supply, there are precautionary measures that can be taken. To begin with, if the fish are properly cooled at the time they are placed in the flumes, they themselves will help reduce the water temperature. In some plants the ice used for holding the fish in the bins or boxes is put into the flumes along with the fish. This helps to keep the water temperature down. But an even more important precaution is to keep to a minimum the time the fish are in the flumes and to have a sufficient flow of water to prevent stagnation.

Deterioration During Stoppages

It is also possible for a certain amount of deterioration to take place in the fillets between the time they are cut until the time they are packed. This occurs during lengthy work stoppages when the fillets are left either in the flumes or on the conveyor belts or cutting boards. If the fillets are sitting on very heavily contamined surfaces they may after a few hours develop an unpleasant odour on the under surface. But more likely the exposed fillets will deteriorate in texture and become soft. For these reasons clause 5-4-4 states that during work stoppages fish should not remain on the filleting line or weighing table for more than 75 minutes. If the unchilled fillets are in lukewarm flumes, or on very heavily contamined surfaces, even a 75-minute delay might seriously injure them. On the other hand, this is quite a safe limit for stoppages when the fillets are on clean surfaces, or in water where the temperature is not allowed to rise above 55°F. The most objectionable thing about long delays on the cutting line is not immediate deterioration of the fish, but that it may allow a serious increase to occur in the number of spoilage bacteria on the fillets. This is especially bad if the fish are to be shipped unfrozen, as it cuts down their subsequent keeping time.

Once the fillets are packaged it is necessary to chill or to freeze them as soon as possible. Clause 5-4-5 states that when they are to be frozen this holding period should not be more than 3 hours. It does not state at what temperature they should be during this holding period. Under any condition the temperature of the fish should not be permitted to rise above 50°F. If the fillets are to be shipped unfrozen the sooner they are chilled after leaving the packing lines the better. A few hours of neglect at this point may cut several days off the keeping time of the fish.

The Problem of High Bacterial Counts

The problem of high bacterial counts on fillets is one of the most perplexing things that a plant foreman has to face. These organisms are so small that they cannot be seen without the aid of a microscope. The result is that fish processors have a tendency to look for some type of magic formula to solve their problem. This may consist of the use of stainless steel or other non-corrosive metals to replace wooden surfaces; it may be the use of the so-called sanitary mechanical conveyors; it may be the replacement of conveyor belts with a system of fluming the fish in flowing water; or it may be the installation of in-plant chlorination.

Modern sanitary equipment for processing and conveying fish is to be heartily recommended. But such equipment in itself will not necessarily result in low bacterial counts. The first essential is a knowledge of the fundamentals of plant sanitation. In order to take the most advantage of modern equipment the foreman must know where the bacteria come from, how they get onto the cutting line; where they may multiply and how they may be eliminated. The first thing is that those in charge of the fish processing become thoroughly familiar with the essential nature of the problems involved so that they can make the most intelligent use of the equipment that is available.

Stated briefly, the fundamental principles underlying the problem of bacteria contamination of fillets are as follows:

If the fish are of Grade I quality there will be only a very small number of bacteria in the flesh at the time the gutted fish reach the cutting table. There may be, and usually are, very large numbers of bacteria in the surface slime, in the gills and in the gut cavity. Generally the longer the fish have been stored in the vessel, the larger the number of bacteria on their surfaces. Therefore, almost all the bacteria that are present on the fillets at the time they are ready for packaging, have either been transferred from the surfaces of the gutted fish or picked up from any of the many surfaces that the fish contact as they pass along the cutting and packing lines.

Practice Differs From Theory

It is theoretically possible to cut almost sterile fillets from surface contaminated round or gutted fish. In practice it is impossible. There will always be some bacteria carried over from the surface of the round fish to the surface of the fillet. Let us suppose that sterile fillets could be cut from the round fish. They would not remain free from bacteria very long. Every surface that they touch as they pass along the cutting table is a potential contributer of bacteria. The problem therefore of producing a low-count fillet is to reduce to a minimum the number of bacteria transferred to it from the round fish and the bacteria picked up by the fillet from the cutting-line surfaces. Essentially these two are the same because the bacteria from the surface of the round fish are transformed to the fillet via the surfaces of the knives, the cutter's hands and the surface of the cutting boards.

Before going into details there is one more generality that should be pointed out. There is a considerable difference between the problem of the initial cleaning of a cutting line and that of keeping it clean all day long while the line is in operation. It is not enough to make a cleanup once or twice a day if the line is going to be immediately recontaminated by a continuous procession of unwashed, slimy, bacteria-laden fish from the vessels.

Methods of Reducing Bacteria on Fillets

(a) *Initial Washing of Round or Gutted Fish.* The round fish themselves are by far the most important source of the spoilage bacteria entering the plant. Many of these bacteria

are in the surface slime, as well as in the loose blood and juices in gut and gill areas. Washing the fish does the double job of removing the slime as well as greatly reducing the number of bacteria. This slime not only harbours bacteria, but it also protects bacteria against disinfectants and provides a splendid medium for continued growth, on cutting boards and other plant surfaces. And incidentally well washed, de-slimed fish are more easily handled by the cutters. There are many ways to wash fish and the method selected must be determined by the facilities that are available and the amount of fish to be washed. The essential thing is to wash them — even if it is only a simple spraying.

(b) *Water Fluming.* Some operators believe that if fish are flumed into a cutting line by means of running water it is not necessary to wash them. If sufficient water is used, and if there is a free flow of water through the fish in the flume, then pre-washing, although still desirable, is not so essential. However, if the flow of water is small, and particularly if the fish are allowed to jam up in the flume, then water fluming is not a substitute for washing. Under these conditions the bacterial content of the water soon builds up into millions per cubic centimeter (cc.) and every time the cutter lifts a dripping fish onto the table he adds another load of spoilage bacteria. If the water flows freely through the fish most of these organisms go down the drain. Water fluming has many advantages but like anything else it can be misused and often is.

(c) *The point of next importance is to prevent the transfer of bacteria from the skin side of the fillet to the freshly exposed, cut surfaces.*

It must be remembered that no matter how well a fish is washed there are still considerable numbers of bacteria adhering to the skin and scales. There are several places where this transfer can take place. The cutting boards on the filleting table are continually being contaminated by the surface of the round fish. If the cutter allows the freshly cut surface of the fish to come in contact with the board it will pick up very large numbers of bacteria. Usually this is avoided by experienced cutters. The skin-on fillets are now placed on a belt or tossed into a water flume for conveying to the skinning table. A careful cutter will place the fillet skin down on the belt and will not let the fillets be piled one on top of another. But it so often happens that not only are fillets piled one on top of another, but they are also allowed to jam up in huge piles at the end of the conveyor belt. This of course is an ideal way of transferring the bacteria from the skin side onto the cut surfaces.

A water flumed conveyor for skin-on fillets creates some of the same difficulties that are met with in using water to convey the whole fish. If the water runs freely and the fillets are not allowed to jam up in the flume, it is reasonably satisfactory. But if the fillets are all jammed up at the end of the flume the water itself soon becomes heavily contaminated and there is ample opportunity for direct transfer of bacteria from the skin surfaces to any cut surface with which they are in contact.

The next point of danger is the skinning table. Of necessity all fillets are placed skin side down on the

BOOK SUMMARY OF SPOILAGE PROBLEMS IN FRESH FISH PRODUCTION

The following summary can be found in "World Fisheries Abstracts" published by the Food and Agricultural Organization of the United Nations (FAO), Fisheries Division, Rome, Italy. These abstracts are published as a service to those interested readers who selected titles from the listings published in the November 1959 issue of CF.

PLANT SANITATION. *Circular No. 22* (Fish Inspection Laboratory, Halifax, Nova Scotia, Canada), 1950. 7 pp. mimeo. In English.

Plant sanitation is important in fish canneries to avoid bacterial loads on the raw material that might endanger proper commercial sterilization, but it is especially vital in the production of lobster meat and shucked scallops, clams, oysters, etc., as these products may not undergo any further heat treatment before consumption.

Plant sanitation is achieved by two main operations: cleansing and sanitizing. The cleansing includes the complete removal of visible dirt by means of a preliminary hosing, followed by a scrubbing of the working surfaces with warm water (around 130 degrees F.) and a cleansing agent (soap, detergent, or wetting agent), and finally by rinsing with boiling water. The heat allows metal surfaces to dry rapidly, thus preventing rusting.

Sanitizing involves the sterilization of working surfaces and utensils after they have been cleaned. The latter may be sterilized in a retort (20 minutes at 250 degrees F.), in flowing steam in a steam box, by immersion into boiling water or into a chlorine solution (3 to 5 minutes in a solution containing 100 p.p.m. available chlorine). Sterilization of working surfaces (tables, equipment) and floors, walls, drains, etc., can be achieved by use of a chlorine solution of the same strength, or usually less effectively, by hosing with live steam.

Chlorine preparations should be stored in tightly stoppered, brown glass, or other opaque containers, in a dark, dry and cool place. They should be prepared daily according to manufacturer's directions, either from dry, powdered chlorine compounds or from liquid hypochlorite solutions.

The problems of the personal hygiene of the plant's employees and of general sanitation, including cleanliness of boats, trucks, wharves, stages, bait houses, plant surroundings, offal removal, and water supply are also discussed briefly. A guide to daily plant sanitation is given:

Tables: A.M. Before start of work, rinse down with chlorine solution (freshly prepared: strength 100 p.p.m.).

Noon. Give a thorough washing. *P.M.* Give thorough washing and hot water rinse.

Utensils: Including shucking knives, washing and draining pans or trays, etc.

A.M. Sterilize prior to use. *Noon.* Give a thorough washing.

P.M. Give a thorough washing and hot water rinse.

Floors: Noon. Wash down with pressure hose, scrub clean with push broom and treat with chlorine solution remaining after day's use.

Worker's hands: Wash thoroughly prior to starting work and after every break in work regardless of cause. Sterilization of equipment should take place at least once each work day; more frequent treatment is advisable.

skinning boards. The skinning boards are therefore being continuously contaminated. Some skinners cut the skin from the fillet and then pull it out from under, allowing the freshly exposed surface to come directly in contact with the cutting board. When this occurs the number of bacteria on the fillet usually increases by 80 to 90 per cent. There are two ways to avoid this. The skinner can cut the skin from the fillet and let the skin remain on the board under the fillet; with a little practice he can then lift the fillet with his knife onto the conveyor without letting it come in contact with the board.

Some mechanical skinners will separate the skin from the fillet without transferring many bacteria to the freshly cut surface. But, as so often happens, this advantage is completely eliminated by allowing the fillets to heap up in a soggy pile immediately before they enter the skinning machine.

(d) *Brines and Preservative Solutions.* Almost all fillets are passed through weak brines or preservative solutions. Plant operators sometimes have mistaken ideas that dipping tanks and dipping solutions need no special care because they contain materials that are purposely used to retard bacterial growth. Unfortunately this is a mistaken conception. In the strengths being used, the antibiotics retard bacterial growth but they do not kill bacteria. If care is not taken, these dipping tanks and solution can build up unbelievably high concentrations of bacteria. In one instance the preservative solution in a dipping tank contained 50,000,000 bacteria per cc. and frequently counts of 1,000,000 or more are encountered. This can be almost entirely eliminated by care in properly draining, flushing and cleaning the tanks and cleaning the conveyor equipment used for carrying the fillets through the solution.

(e) *Candling Tables.* From the standpoint of sanitation the candling tables present some special problems. Unlike the cutting boards, they are non-porous and hard surfaced. For this reason they can easily be cleaned and disinfected. On the other hand, in practice, they are rarely cleaned and are almost always covered with a coating of slime. And this is the one place in the cutting line where the temperature increases to a point where the bacteria undergo their maximum rate of reproduction. Where ordinary electric bulbs are used for illumination the temperature of the glass often reaches 70 to 80°F. This is ideal for rapid bacterial multiplication. Fortunately this is another problem that is relatively easily solved. Either a continuous spray of water or frequent washing followed by the use of a good disinfectant keeps the candling table in a condition where the fillets can be passed over it without any significant increase in bacterial content. A somewhat similar result is obtained by conveying the fillets to the candling table by means of a water flume. The water carried onto the table by each individual fillet helps wash off the bacteria from the glass. Care must be taken in this case to see that such fluming does not interfere, by dilution, with any previous preservative dip.

(f) *In-plant Chlorination.* The question is frequently asked: will in-plant chlorination significantly reduce the bacterial count on fillets? The answer is that if used intelligently chlorination, like water fluming and mechanical skinners, can be used to considerable advantage. But one must know where it can be used and what are its limitations. There are two basic things that should be remembered by those considering the use of chlorine in a fish plant. (1) *In the absence of organic matter* chlorine is a good bacteria killer. (2) Chlorine inactivates the antibiotics used for preserving fish.

Proper Use of Chlorine

In clean, clear water, a half a part per million (ppm) of chlorine will kill most bacteria — because there is relatively little organic matter. However, as the organic content of the water is increased a corresponding increase in the chlorine is required to do the same job. In polluted harbour water, or in lake or river water containing considerable amounts of organic matter, the chlorine content must be increased to somewhere between 5 and 15 ppm. If we add fish slime and small particles of fish waste to the water, still stronger solutions (25 to 100 ppm) are required to kill the bacteria. And it is almost useless to try to kill the bacteria in or on round fish or fillets by dipping them in chlorine solutions. So much chlorine would be needed to do an efficient job that it would give the fish an unpleasant flavour.

Similarly, if we wish to destroy the bacteria in slime-soaked cutting boards or in dirty wooden fish boxes we must use several thousand parts per million of chlorine and leave them in the solution for several hours.

On the other hand there is much to be said for the installation of chlorine equipment that will deliver a flow of water containing 25 or 50 ppm. in the cutting rooms of the plant. Chlorine cuts slime and increases cleaning efficiency. If nonporous troughs, tanks, conveyer belts and other surfaces are first freed from slime, fish "gunk" and other organic matter, then, and then only, chlorine at this concentration actively kills the remaining bacteria. Chlorine is also a deodorizer and removes most of the unpleasant odours that are so often found where fresh fish is being processed. In some plants where fish cutters have been accustomed to the odour of decaying fish slime, they resent the odour of chlorine. They say it reminds them of "chloride of lime treated country outhouses". This is just a matter of association. In one of the best equipped and cleanest fish plants in Canada (in British Columbia, incidentally) they have just such a system of in-plant chlorination and it is used for periodic flushing down of the cutting lines and floors after they have been washed. The workers in this plant associate the chlorine odour with clean sanitary conditions in the plant.

Many other suggestions might be given that would vary with the plant and the type of equipment being used. Any place where fish slime or fish juice is allowed to collect, bacteria are sure to grow. Any surface that the fillets touch or any solutions that the fish are passed through should be looked at with suspicion. And lastly, if the plant foreman or the fishery officer who is helping in a clean-up campaign can obtain the services of a bacteriologist or technician who can check the numbers of bacteria on the fillets and at various places in the cutting line, the work will become easier and the results will be more clearly demonstrated.

Effects of Heat Transfer On Quality of Fish

The effects of temperature on fish quality should now be quite well understood, in the light of what has already been said about bacterial growth. Temperature is important also from the chemical deterioration standpoint since such deterioration will increase rapidly with temperature rise, and conversely decrease with temperature drop. Of course the extent of both bacterial and chemical deterioration will depend upon the time factor as has been emphasized already, but this time

factor requires a little more study.

This is not a simple matter when we are dealing with heat transfer and the cooling or heating of fishery products. Cooling problems may appear to be quite simple, but when we try to determine theoretically the time required by a body to reach a given temperature under rather simple conditions, we encounter complicated mathematics; and complicated problems often defy mathematical treatment completely. However, the purpose here is to throw some light on the time factor involved in chilling fish, and thereby give us a better understanding of its relationship to quality where temperature is concerned.

Heat is transferred by three means: *radiation, conduction,* and *convection. Radiation* occurs for practical purposes in fish handling only on exposure to sunlight and its effects are well known.

Heat transfer by *conduction* is perhaps most important in fish handling, and this is governed by Fourier's Law:

$$q = KA \frac{dt}{dL}$$

or more simply for our purposes it is integrated to

$$q = KA \frac{t_1 - t_2}{L}$$

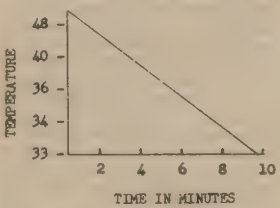
meaning that a quantity of heat q is transferred through a slab of area A and thickness L, having a thermal conductivity K and temperatures of t_1 and t_2 existing at its two surfaces. In the terms with which we are all familiar, this means that we can cool or heat more rapidly by increasing the surface area, reducing the thickness and employing the greatest possible temperature difference. However, there are practical limitations to how far we can go in employing these factors in achieving rapid cooling. For reasons of economy we reduce A and increase L to obtain more economical containers or more effectively use floor area in storage. In the use of ice the freezing point puts physical limits on the increase of $t_1 - t_2$ and the use of mechanical refrigeration has a practical limit because of the reduced efficiency of refrigeration at relatively low temperatures.

We shall take an example with which we are all familiar to demonstrate how heat transfer by conduction can affect quality; that is the example of comparatively warm fish piled on ice in a bin and adequately topped with ice.

First, with regard to the height of the pile, we shall examine our equation

$$q = KA \frac{t_1 - t_2}{L}$$

We can see that the rate of heat removal decreases with increase of L or height. However, the quantity of fish and subsequently the amount of heat increases with L, thus the time for cooling increases with L^2. In examining $t_1 - t_2$ we find that this difference becomes smaller as the temperature of the fish drops; consequently, the rate of cooling decreases as we come near our desired final temperature. This temperature relation can best be shown by the following graph of the cooling of a 4"-thick slab of fish.



Graph 1 — Cooling 4" Slab

From this graph, which shows that a 4" thickness required approximately 2 hours to reach 38°F, a temperature where spoilage is still progressing at roughly twice the rate it does at 32°F, we can now see that thicker piles would not cool at their centers to any appreciable extent when we apply the L^2 relationship previously discussed. For example, a 2-ft. pile having L six times as great would require 36 times the time or 72 hours to reach the same temperatures.

Convection, the third means of heat transfer, has its effect on fish quality also. To understand it we must obtain the concept of surface conductance or film coefficient. It is defined as the quantity of heat/hr. transferred to a square foot of surface from a fluid per °F difference or,

$$q = h\,A(t_1 - t_2)$$

where h is the surface conductance, q = rate of heat transfer, A = area, and $t_1 - t_2$ = the temperature difference. The surface conductance depends on the physical nature of the body in question and its relative velocity, and to some small extent on the physical nature of the surface. Some representative examples of surface conductances are given in Table 1.

TABLE 1

Air on wall surface, natural convection	1.65
Air on wall surface, 15-m.p.h. wind	6.00
Air forced across 1-inch tubes at 10 f.p.s.	7
Water on surface, natural convection	30
Water at 4 f.p.s. in 1-inch pipe	930
Condensing steam	300-5000

Noting the very low value, 1.65 Btu/ft² hr/°F for still air, let us consider the example of a 10-lb. box of fillets at 50°F being placed in a cold room at 32°F in a single layer with top and bottom surfaces exposed to the air. The dimensions of the package are 12 x 12 x 12 in., its total surface area exposed being 2 ft².

From our formula,

$$q = h\,A(t_1 - t_2)\ \text{Btu/hr.}$$

Thus, in 1 hr. the heat removed

$$Q = 1.65(2)\ (50\text{-}32) = 59.4$$

and the drop in average temperature is:

$$\Delta t = \frac{Q}{WC} = \frac{59.4}{10(.85)} = 70^{\circ}\text{F.}$$

where C, the specific heat of fish is taken as 0.85. Because t_1, the surface

*Footnote

$$q = hA(t_1 - t_2)$$

$$q = -hA(t - ta)$$

$$q\,d\theta = cw\,dt$$

$$cw\,dt = -hA(t - ta)\,d\theta$$

$$\frac{dt}{t - ta} = -\frac{hA}{cw}\theta$$

$$\ln\left[t - ta\right]_{t_o}^{t} = -\frac{hA}{cw}\theta$$

$$\ln\frac{t - 32}{50 - 32} = -\frac{1 - 65(2)}{0.85(10)}\theta$$

$$\ln\frac{t - 32}{18} = -0.389\,\theta$$

For θ = 1 hour

$$\ln\frac{t - 32}{18} = -0.389$$

$$\frac{t - 32}{18} = 0.679$$

$$t - 32 = 0.679(18) = 12.2$$

$$t = 32 + 12.2 = 44.2°\text{F}$$

$$\Delta t = 50 - 44.2 = 5.8°\text{F}$$

BOOK SUMMARY

The following summary can be found in "World Fisheries Abstracts" published by the Food and Agricultural Organization of the United Nations (FAO), Fisheries Division, Rome, Italy. This abstract is published as a service to those interested readers who selected this title from the listings published in the November 1959 issue of CF.

CHLORINATION IN FISH PLANTS. H. P. Dussault. *Progress Reports of the Atlantic Coast Stations* (Fisheries Research Board of Canada). No. 66, p. 13, Sept. 1957. 2 pp. in English.

Chlorine is one of the most effective sanitizing agents used in modern food plants. It is doubtful, however, that the full importance of the value of chlorination is adequately understood by fish plant personnel. Recently, some of the members of the fishing industry have studied chlorination as a means of solving some of their problems in sanitation. Automatic chlorination systems have been installed in some fish plants. The advantages, as gathered from a study of plant problems made in 1954, are presented. This study is based on two plants that produce frozen fish fillets and blocks for the preparation of fish sticks.

Source of pollution. Both the fresh and sea water used in plant no. 1 contained significant counts of coliform bacteria — over 1,800 coliforms/100 ml. of water. In plant no. 2, only the sea water was found to be polluted, owing to the plant location on the shore of a harbour into which raw sewage was emptied constantly. In both of these plants, coliform bacteria were found along the filleting lines and on the hands of some of the workers who were handling the fillets. Brines for dipping fillets showed significant numbers of coliform bacteria that had accumulated, owing to the constant passage of large quantities of fillets through the brine. In some instances, coliform bactera were found on the packaged fillets, both fresh and frozen. These products were a menace to the health of consumers. Fish slime, which is an excellent culture media for bacteria and which is difficult to remove, had accumulated on everything with which the fish came into contact along the filleting lines. A strong and unpleasant fishy odour prevailed throughout these two plants.

To overcome these conditions the author recommended the installation of automatic chlorinators that add chlorine directly to the incoming water so as to maintain a residual concentration of 3 to 5 p.p.m. He also recommended that, during cleaning periods, the chlorine concentration be increased to 30 p.p.m. and that all workers dip their hands in a solution containing 100 p.p.m. of chlorine before beginning work and particularly after each visit to the lavatory.

The author's recommendations were followed. On a 2nd visit to each of these 2 plants, all sources of water and even water from the polluted harbour were found to be absolutely free from coliform bacteria. As a consequence, there was almost complete absence of these bacteria on the hands of the workers, on the filleting lines, in the brine baskets and in the finished product. Chlorine proved to be an excellent bactericide and a most effective cleaning and de-sliming agent. Fillet boards were free of slime and cement floors were less slippery as a result of slime removal. In addition the fishy odour within the plant had disappeared and had been replaced by the fresh odour of chlorinated water.

The plant managers were very pleased with the simplicity of automatic chlorination and with the results obtained. Workers were pleased by the improved sanitary conditions within the plant and morale was at a high level.

This difference between freshly caught fish and iced fish was clearly demonstrated a few years ago in some trials at two different fish plants. In one plant all fish were transported in boxes and dumped directly on the conveyor belt. At the other plant the fish were transported in flumes containing running water. After the fourth day, the number of bacteria on fillets from fish, flumed in water, decreased sharply. The decrease resulted from the slime becoming easier to remove by washing.

temperature, is changing, this result is rather high; the true average temperature drop obtained by more involved mathematical treatment is about 5:8°F. *

The progress of cooling is shown below in Table II.

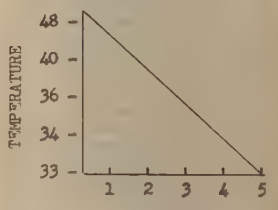
TABLE II

θ	t	$\frac{1}{\Delta t}$
0 hr.	50°F	0°F.
1	44.2	5.8
2	40.2	9.8
3	37.7	12.3
4	35.8	14.2
5	34.6	15.4
6	33.7	16.3
7	33.1	16.9

Had the boxes been packed in ice where there would be no effect of surface conductance, the average temperature drop would be 17°F or within 1° of ice temperature.

From this example we can see the limiting effect of surface conductance on cooling and realize that the use of holding rooms has no useful function in the cooling of fish. Their only use is in holding fish already cooled to proper temperature by other methods.

Returning to our examples of surface conductance coefficients we will note that water has very high values beginning at about 0 Btu/hr/ft²/°F and increasing rapidly with agitation. As a result of this high value and a small thickness, small fillets will gain or lose heat rapidly in water as occurs in fluming, dipping and washing. The second graph shows an example of a typical fillet of ½-inch average thickness at 50°F placed in ice water. From this example it can be seen that fillets will gain or lose about 10°F in 3 minutes. In the case of heat gain, the previous example has shown that these fillets now placed in storage will remain at those elevated temperatures for long periods unless frozen. To avoid this condition all water treatment of fillets must be at low temperature or be followed by ice water dipping to remove the heat gained.



Graph 2 — Cooling of Fillets

Freezing Fish

The freezing of fish, one might say, involves converting the fluid portions of fish muscle from the liquid to the solid phase (ice) and although this entails merely the extraction of heat, the rate at which the heat is extracted has a great deal to do with the properties of the frozen product.

Experiments have shown that when heat is extracted very rapidly from fish in the freezing process, the ice crystals formed in the fish muscle are relatively small whereas if the heat is extracted rather slowly the ice crystals are correspondingly larger. Controlled taste-panel tests carried out with both rapidly and slowly frozen samples of fish muscle have shown only very slight differences in the taste reactions, if the samples are consumed almost at once. However, if the storage factor is introduced it was found that the rapidly frozen samples (those with the smaller ice crystals) retained high quality for longer periods than did the slowly frozen samples. Controlled biochemical tests have supported the taste panel tests, consequently rapid freezing is strongly recommended by technologists.

Many attempts have been made to determine a critical rate of freezing for fish, but so far none has been defined. However, many prolonged storage tests have shown that if the temperature at the centre of the thickest portion of a sample of fish is reduced from +5°F to —5°F in less than say 2 hours, then those samples will retain a satisfactory quality level for 6 months to 1 year, depending on the species of fish and the storage temperature. These tests formed the basis for specifications 5-4-7 of the C.G.S.B. standards for fresh and frozen fish.

Importance of Thickness

The section of this discussion dealing with heat transfer showed the effect of increased thickness of sample (pile of fish in that case) on the rate of change of temperature. The relationship was as the square of the thickness, with other conditions kept constant. That means that if one sample is twice as thick as another sample it will require 2^2 or 4 times the time to "freeze" the thicker sample, other factors remaining equal. Actually all other factors in the case of freezing do not remain the same because the change in phase (liquid to solid) changes the conductivity and specific heat of the fish muscle, but practical effects are relatively insignificant with fish, so the square relationship between thickness and temperature change is sufficiently accurate for practical purposes.

This would mean that if a processor wished to freeze 2-inch thick blocks of fillets rather than one-inch thick blocks as referred to in specification 5-4-7, the facilities that meet that specification would require 8 hours to "freeze" the thicker blocks.

These observations will emphasize the importance of thickness when dealing with freezing as well as with chilling of fish. They will also indicate the difficulty encountered in freezing whole fish, particularly large (thick) ones.

Freezing Done In Air

Specification 5-4-8 deals with whole or dressed fish and states the requirements when freezing is done in air. Here no freezing time is specified because the time factor will vary with the thickness of the different specimens of fish as already explained, so the conditions of the air are given. Under these circumstances the temperature of the air and its velocity are very important, since air is a relatively poor conductor of heat and must be kept moving rather rapidly in order to give a satisfactory surface conductance factor, as indicated in Table I. Air is not only a poor conductor, it has also a low heat capacity when compared with liquids and solids; consequently, a small amount of heat will change its temperature appreciably. When designing an air-blast type of fish freezer, therefore, particular attention must be given to the velocity of the air over the fish to be frozen and over the refrigeration coils that do the cooling. In order to transfer the heat from the former to the latter vast amounts of air must be circulated in the least possible time within practical limits.

To give some idea of quantities involved in the freezing process it might be worth while considering a few data in a simplified illustration.

In reducing the temperature of one pound of fish muscle from say 50° to —5°F and assuming that (a) the muscle is composed of 80 per cent water and has a specific heat in the unfrozen state of 0.85 (the factor used in the chilling problem above), (b) the latent heat of fusion = 80% of that of water, namely 0.8 x 144 = 115.2 Btu/lb. fish, (c) all the freezing takes place at 30°F, (d) the specific heat of the frozen muscle is ½ that of the unfrozen muscle, namely 0.425, then the amount of heat to be extracted will be the sum of the cooling to chill from 50° to 30° = 0.85 x 20 or 17.0 Btu., plus the heat to freeze = 115.2 Btu., plus the heat to further cool the frozen muscle from 30°F to —5 = 0.425 x 35° or 14.9 Btu., giving a total of 147.2 Btu. for the pound of muscle under consideration.

The specific heat of air at constant pressure is approximately 0.2375. If we assume a 10°F temperature change in the air passing over the fish, then the amount of air required to freeze the fish will be 147.2 ÷ 10 x 0.2375, or 62 pounds approximately. Since the specific volume of the air under the conditions required for our hypothetical case will be roughly 11.2 cubic feet (cu. ft.) then the amount of air required to be circulated will be 62 x 11.2 = 694.2 cu. ft./pound of fish frozen.

If the 147.2 Btu. given up by the hypothetical pound of fish are ultimately absorbed by liquid ammonia (a common refrigerant used for freezing these days) which will thereby be evaporated, it will be found that something like 1/3 of a pound of ammonia will be required. So we see that to transfer this heat from 1 pound of fish to the 1/3 pound of ammonia we are obliged to use 62 pounds of air. Obviously our choice of heat carrier is a very poor one.

In the contact or plate-type of freezers air as a heat carrier is eliminated entirely and because of their relatively high effectiveness they are strongly recommended for all packaged fish. They are not adaptable to freezing whole or dressed fish, however; consequently we shall probably continue to have air-blast type freezers with us until all commodities are frozen in the packaged form.

In recapitulation the following points have been made:

- (1) Fish should be processed as soon after capture as possible.
- (2) Immediately after capture fish should be held at a temperature at or near their freezing point.
- (3) All handling and processing procedures should stress speed, cleanliness and gentleness.
- (4) Liquid cooling, conveying and treatment media have relatively high conductivity therefore their temperature should be maintained as close to the freezing point of the fish as practicable.
- (5) Air is a relatively poor conductor of heat, therefore in a quiescent state it is not very effective in extracting heat from fish.
- (6) The contact types of freezers are very effective in freezing packaged fish.

Chlorine Affects Antibiotics!

by Charles H. Castell
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Chlorine *and* Antibiotics are useful partners in a fish processing plant. The one takes over where the other leaves off, BUT . . . . .

When they come in contact, Chlorine *destroys* Antibiotics!

How can you prevent this?

Quite simply, states the author of this paper. To avoid this destruction and ensure that the product enjoys the preservative value of antibiotics, the antibiotic dipping solution must be prepared with *de*-chlorinated water.

How this can be done in a jiffy, without any additional equipment or specialised knowledge, is described by the author.

T THE PRESENT TIME there are two types of chemical compounds that are widely used in ian fish plants for reducing bac- contamination and for increas- the keeping time of the fillets. are chlorine, or compounds that e chlorine, and the broad spec- antibiotics (Acronize and Bio-). In the processing line chlorine antibiotics perform different jobs they will not replace each other. lorine is used primarily as a dis- tant and at times it has the addi- l function of being a deodorant. municipal water supplies that are in fish plants are treated with ine gas to destroy the harmful ria. In addition to this, plants may be equipped with a separate and strongly chlorinated water sys- that can be used for periodic wash- and rinsing of the tables, belts, ng boards and other surfaces where accumulates and bacteria develop. the absence of organic matter, ine is one of our best bacteria rs For this reason it is almost ersally used in destroying undesir- bacteria in our water supplies. As amount of organic matter increases germ-killing action of the chlorine eases. For example, between 0.3 1.0 parts per million (p.p.m.) rine will kill almost all harmful bacteria in very clear water. In polluted harbour water or in lake or river waters containing considerable organic matter, the chlorine content must be increased to somewhere between 5 and 15 p.p.m. to do the same job. If fish slime and small particles of fish waste are added to the water, the germ-killing action of the chlorine is still further reduced. And it takes a very strong solution of chlorine (500 to several thousand p.p.m.) to kill bacteria that are imbedded in the surface of fillets or in slime-soaked cutting boards. For this reason the proper and most efficient use of chlorine in a fish plant is for destroying bacteria before they get onto the fish: it kills bacteria in the water supply and slightly stronger solutions, in conjunction with thorough scrubbing and cleaning, will reduce the number of bacteria on various surfaces of the cutting line. There is a very definite place for chlorination in a fish processing plant.

BUT CHLORINE IS NOT A GOOD FISH PRESERVATIVE. In order to function satisfactorily as a "dip", a chlorine solution would have to be so strong that it would impart unpleasant flavours and might also discolour the surface of the fillets.

The antibiotics such as "Acronize" or "Biostat" are not primarily germ killers like chlorine. The simplest way of describing their action is that they paralyze the bacteria. Without killing them, they stop or greatly retard the growth of the bacteria. This in turn stops or retards the spoilage due to bacterial activity. Unlike chlorine, the antibiotics are affected very little by organic matter. This makes these particular antibiotics almost ideal preservatives. Their job is to act on the bacteria *after they have got on the fillets*, and this they do without imparting any unpleasant properties to the fish.

From this it can be seen that chlorination and antibiotic treatment make a very useful combination in a fish processing plant. One takes over where the other leaves off.

The Action of Chlorine on Antibiotics

The useful partnership of chlorine and antibiotic sometimes runs into serious trouble. This occurs at the moment we let the two partners come into direct contact with each other.

Chlorine is a very active and often a very destructive chemical. We use it because it so quickly destroys bacteria. We also know that chlorine corrodes metals and attacks many other chemicals. What will chlorine do to our fish preserving antibiotics? To answer this question we ran a series of experiments, the results of which are reported here.

In the first preliminary experiment a solution of "Acronize" was made up containing 10 p.p.m. CTC. (This is the usual concentration for "Acronize" when it is to be used for dipping fish fillets). To this solution sufficient sodium hypochlorite was added to give a theoretical concentration of 10 p.p.m. available chlorine. After standing 10 minutes the solution was tested to determine the CTC content. It was found

ronize" is a trade name for an anti- produced by the American Cyanamide pany. The active ingredient is chlor- cycline (CTC).
stat" is a somewhat similar product uced by Chas. Pfizer and Company. Its e ingredient is oxytetracycline (OTC).

oduced by permission from the Canadian Fisherman, Vol. 47, 2, pp. 37, 39, February 1960.

that there was none present! It was also found that most of the chlorine had also disappeared! Apparently the partners did not get on well together.

The next experiment was a little more elaborate. The purpose was to see if a relationship could be found showing the amount of chlorine that would be required to destroy a given concentration of CTC.

Using the City of Halifax tap water, an "Acronize" solution containing 15 p.p.m. CTC was prepared and distributed among 8 glass-lined tanks. Hypochlorite was added to give 0, 0.5, 1, 2, 3, 4, 5 and 10 p.p.m. available chlorine. After stirring vigorously these solutions were left standing for 10 minutes and then tested for CTC. The results are shown in Figure 1. It may be seen that the amount of CTC destroyed increased as the concentration of the added chlorine was increased. Even 1/2 p.p.m. chlorine destroyed some CTC and 5 p.p.m. chlorine reduced the initial CTC concentration by two thirds.

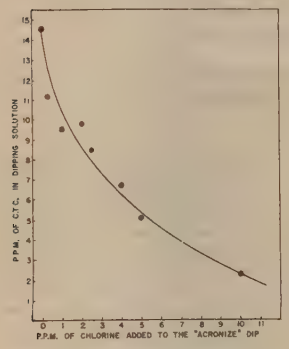

Fig. 1. CTC content of "Acronize" solutions 10 minutes after adding various concentrations of chlorine. Initially all the Acronize solutions contained 14.6 ppm CTC.

Many other experiments were carried out using various amounts of CTC and chlorine. These will be published in detail in the Journal of the Fisheries Research Board of Canada. However, they all say the same thing: chlorine attacks and destroys CTC.

Chlorine Affects CTC Dips

The obvious inference from these results is:— don't use water containing chlorine for the preparation of "Acronize" dipping solution.

Most city water supplies are treated so as to have somewhere about 0.3 to 0.5 p.p.m. available chlorine. As the water flows through the mains much of this chlorine is used up and by the time it reaches the fish plant there may be only a fraction of the initial content. This normally chlorinated water supply is not strong enough to seriously effect the CTC. Following storms or for certain other reasons, the engineers may occasionally increase the chlorine content above normal. Usually this can be recognized by observing a chlorine odour as the water comes from the tap. In this case there may be sufficient to interfere with the CTC in a dipping solution. More serious, however, are the instances where the plants have their own chlorinators — adding 5 or 10 p.p.m. chlorine to their water supplies. This will drastically cut down the CTC.

It is best to make up the CTC dipping solution in clean pure water that has not been chlorinated or in a weakly chlorinated city water. If some reason or other nothing but ch inated water is available, it is a s ple matter to dissipate the chlorine adding some suitable organic mat of course this organic matter must clean and absolutely harmless. Ide suited for this is the fish itself. S pose we wish to make up 10 gal of dipping solution using a water s ply that contains 5 p.p.m. chlorine we immerse 5 lb. of fillets in the wa for 20 seconds and then let the wa stand for 15 minutes, almost all chlorine will be used up. It can t be used for preparing the CTC solut

NOTES ON SOME POLYCHAETA FROM THE WEST COAST OF MEXICO, PANAMA, AND CALIFORNIA[1]

E. Berkeley and C. Berkeley

Abstract

Notes on 13 species of Polychaeta selected from collections made on, or near, the coasts of California and Mexico during 1958 and 1959 are recorded. Of these one (*Eurythoe complanata* var. *mexicana*) is a new variety, three (*Paraeurythoe americana*, *Halla parthenopeia*, *Asychis amphiglypta*) are new records for the eastern Pacific, three (*Perinereis obfuscata*, *Nereis oligohalina*, *Polyophthalmus pictus*) involve new records of swarming forms, and two (*Glycera robusta*, *Aphrodite falcifera*) are extensions of distribution. The remainder deal with species having miscellaneous features of some other interest.

Introduction

We reported in 1958 (5) on a collection of Polychaeta from the northeast Pacific south of latitude 32° made by Mr. H. L. Klawe of the Inter-American Tuna Commission in the years 1955, 1956, and 1957. During 1958 and 1959 we received further material from the same source, mainly collected in Mexican waters, and during 1959 Mr. H. Arai of the University of California, Los Angeles, has also sent us small collections from the same region, whilst quite recently we have received specimens of a very interesting maldanid from Mr. W. A. Newman, of the University of California, Berkeley. Many of the species in these collections have already been recorded from the region and are fully described elsewhere. The present paper deals only with those items in them which have noteworthy features. The individual collectors are indicated in the text by the initials H. L. K., H. A., and W. A. N., respectively.

Aphroditidae

Aphrodite falcifera Hartman. Hartman (14)

A single example of this species, from San Diego, California (H.L.K.), is 32 mm long and 14 mm wide as preserved, considerably longer than the type, and only previously known, specimen. This was 22 mm long and was recorded from Carros Island, Mexico. Its distribution is now extended northwards.

Polynoidae

Chaetacanthus magnificus (Grube). Seidler (28)

Two specimens, both about 30 mm long and 5 mm wide, taken in dead coral at Zihuatanejo, Guerro Province, Mexico (H.L.K.). Seidler (28) and Hartman (14) regard *Polynoe branchiata* Treadwell (29) as synonymous with *Chaetacanthus magnificus* (Grube) and Hartman adds *Lepidonotus pilosus* Treadwell (31) to the synonymy. The present specimens have been identified

[1]Manuscript received December 24, 1959.
Contribution from the Fisheries Research Board of Canada Biological Station, Nanaimo, B.C.

from a joint consideration of Seidler's and Treadwell's descriptions. The species seems to be somewhat variable. The specimen from Espiritu Santo Island, Mexico, recorded by us (2) as *Lepidonotus pilosus* Treadwell, for instance, having elytra with much less heavy fringes and smaller tubercles than the present examples, so that the dorsum has a much smoother appearance.

Originally described from the West Indies this species has been recorded previously from the eastern Pacific from Ecuador to the Gulf of California (14).

Amphinomidae

? Paraeurythoe americana Hartman. Hartman (18)

A single specimen taken at night at Balboa, Panama (H.L.K.), is a ripe female distended with eggs. It is considerably contracted as preserved, measuring 85 mm long and 5 mm wide and thus small by comparison with the dimensions given by Hartman (18). In this respect and in that, owing to the small size and retraction of the prostomium, we have been unable to determine the presence or absence of a caruncle with certainty, some doubt attaches to our identification, but agreement in all other particulars is close.

P. americana is recorded only from the Gulf of Mexico, but Hartman (18) suggests synonymy with *Eurythoe dubia* Monro (21), which is recorded from the same locality as the present specimen. Our observations lend probability to this synonymy.

Eurythoe complanata (Pallas) var. *mexicana* var. n. Hartman (15) (stem-species)

An anterior portion, about 40 mm long and 5 mm wide, consisting of some 30 segments, and an anal portion, about 32 mm long and 3 mm wide, from San Carlos Bay, Gulf of California (H.A.), agree with this widely distributed species except in one respect. In both specimens most of the notosetae in all the parapodia closely resemble the abnormal form described by us (2, Fig. 1) from a specimen taken at Espiritu Santo Island, Gulf of California, the only difference being that the tips are less expanded. In some parapodia all the smooth notosetae are displaced by these, but a few of the large serrate ones always remain. That they are not worn or broken shafts of normal setae is clear from the fact that many which have not yet penetrated to the exterior can be seen in the notopodial lobe. When we observed these setae first, we regarded their presence as an individual idiosyncrasy of the specimen in which they were found, but the present second case of their occurrence suggests that it has at least varietal significance.

Glycera robusta Ehlers. Ehlers (7), Berkeley and Berkeley (4)

A fine specimen, 600 mm long as preserved, from San Carlos Bay, Gulf of California (H.A.). This appears to be the most southerly station at which this species has been collected on the east coast of the Pacific. It is common farther north. Okuda (24) records it from Nakanoshima, south Japan, in approximately the same latitude as that at which the present specimen was taken.

Nereidae

Nereis (Ceratonereis) tentaculata Kinberg. Hartman (15) (synonymy); Ehlers (8), as *C. mirabilis*; Treadwell (30), as *C. singularis*

Two specimens of the atokous stage of this species from dead coral, from Zihnatanejo, Mexico, and several heteronereids from Le Paz Harbour, Gulf of California (H.L.K.). All the heteronereids except one are males. They are all more or less broken, but specimens can be reconstructed and show three distinct body regions. This is in accordance with Treadwell's description (30) of the heteronereids of *Ceratonereis singularis*, a synonym of the present species. Treadwell finds that the change to the epitokous parapodium occurs after the 16th somite and that the terminal region consists of about 25 somites. In our specimens the change occurs (in males) at the 22nd or 23rd somite and the number of somites in the posterior region is very variable, as many as a hundred or so being present in some individuals. The species is widely distributed in southern latitudes.

Perinereis obfuscata Grube. Monro (20), Rioja (25)

Two heteronereids of this species, one of each sex, from Zihnatanejo, Mexico (H.L.K.). Rioja's description (25) of atokes from Acapulco, Mexico, corresponds closely with the present specimens except in so far as they are modified by heteronereid characters. Monro (20) describes epitokes of both sexes and with these also our specimens are in complete agreement. The male has 14 setigers anterior to the first epitokous parapodium, the female 18.

Nereis oligohalina Rioja. Rioja (26)

Several male heteronereids and a few females from Le Paz Harbour, Gulf of California (H.L.K.). We recorded in our 1958 paper (5) that the former has 17 setigers anterior to the first epitokous parapodium. To this may now be added that there are 22 such setigers in the female.

Eunicidae

Halla parthenopeia (Delle Chiaje). Fauvel (10), Okuda (23), Ehlers (7), as *Cirrobranchia*

A single specimen 100 mm long, 8 mm wide, collected by J. Wintersteen (submitted to us by H.A.), at San Luis Bay, Gulf of California, intertidal, agrees closely with Fauvel's and Ehler's descriptions except in that we have not been able to find the hispid capillary setae described by the former, and the latter omits mention of the bidentate hooded crotchets which Fauvel describes and are present in our specimen. The three tentacles and the first segment are much contracted and withdrawn into the second segment, so that the characteristic tentacular depression can be seen only with difficulty. The body color is a rather dark brown as preserved.

Halla parthenopeia is one of the largest errant polychaetes known, attaining a length of 90 cm, so that the present specimen must be regarded as a very young individual. It has not been recorded previously from the eastern Pacific, but is fairly common in Japan (23).

Spionidae

Scololepis indica Fauvel. Fauvel (12 and 13), Berkeley and Berkeley (3)

A single individual incomplete posteriorly from San Carlos Bay, Gulf of California, intertidal (H.A.), agrees exactly with Fauvel's descriptions. We recorded specimens from Mission Bay, California, in 1941 (3), since which the only notification of its occurrence seems to be that of Day ((6), as *Scolecolepis*) from South Africa. In the example from Mission Bay we found tridentate-hooded crotchets in some parapodia and similar instances are recorded by Day (6). These are not mentioned in the original descriptions and we have found none in the present specimens.

Maldanidae

Clymene (*Euclymene*) *grossa* Baird var. *newporti* Berkeley. Berkeley and Berkeley (3), Ehlers (9) (stem-species)

A single example from Point Loma, California, intertidal (H.L.K.), is noteworthy by reason of its size. It is 200 mm long and 8 mm wide as preserved, considerably larger than any previous record of either the stem-species or of the variety. Of the former the largest known is 125 mm long and 6 mm wide and, of the latter, 70 mm long by 4 mm wide.

Asychis amphiglypta (Ehlers). Ehlers (8), as Maldane; Arwidsson (1); Monro (22)

Many anterior and posterior halves of specimens dredged in 3 to 6 meters in South San Francisco Bay, California (W.A.N.), agree closely with this species as described fully both by Ehlers and Arwidsson. Since all the specimens, except one or two very small ones, are broken we cannot determine a maximum length. The greatest width of the widest fragment is 3 mm as preserved. The species is characterized by having the cephalic border divided by a deep notch on each side into two lobes the edges of which are quite entire, but may be more or less undulate. The nuchal grooves are short and bent into a crescent anteriorly, but the posterior end of the groove is frequently obscure and the grooves then appear as transverse crescents. There is no collar on the first setiger. There is only one preanal segment which is frequently not well defined. The margin of the anal plate of the pygidium is extended and divided into two lobes, the ventral one forming a pocket over the plate, the dorsal extending beyond the anus. The latter lobe has no trace of border filaments such as Monro (22) found in some specimens of the present species and which characterize several other members of the genus.

The species has been taken previously only from subantarctic stations, so that its distribution is considerably extended by the present record. *Asychis maculata* (Kinberg), as briefly described both by Kinberg (19) and Hartman (17), seems to be near *A. amphiglypta*. It is recorded from India.

Opheliidae

Polyophthalmus pictus (Dujardin). Fauvel (11)

Many specimens taken swarming at a light at Zihnatanejo, Mexico (H.L.K.), chiefly males. This widespread species has been recorded in the atokous stage from Acapulco, Mexico (25), from the Gulf of California (27, 3), and from California (14, 16). The epitokous stages are known from the Mediterranean region (11), but have not been recorded previously from the Pacific. They differ from the atokous form in that the setae are considerably elongated in the last five or six setigers and the nephridial openings are dilated.

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COMMERCIAL FISHERIES UTILIZATION

by

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The Inland Commercial Fisheries of Canada are
erally regarded as a relatively small part of the total
adian Commercial Fishery. Canada produces annually
at two billion pounds of fishery products including
ster, oysters and other shell fish. Of this, only
at 6% are fishery products from the inland fisheries.
ever, because the fresh-water fisheries are more
orably situated for marketing their products, the value
the inland commercial catch is a larger proportion of
whole. The annual freshwater catch is valued at
,000,000 landed and $20,000,000 as marketed, or 13% and
% respectively of the values of the total Canadian catch.
terms of people involved, the freshwater fishery is an
n larger fraction, since there are about 16,000 commercial
hermen in our inland fishing, or about 22% of the total
ber of Canadian Commercial Fishermen. Looking at it

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er presented at the Second Alberta Resources Conference
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ources Conference."

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another way, close to one Canadian in every thousand is a fresh-water commercial fisherman, and he is generally the main bread-winner in a family.

Detailed maps of northern Canada show a tremendous number of lakes, particularly in the Pre-Cambrian Shield. Most of the lakes have commercial species of fish in them. However, commercial fisheries are mostly confined to those lakes near roads and railroads. Although not fished commercially, the more remote lakes are an important source of food for men and sleigh dogs in those areas.

The most important species of Canadian fresh-wate fish is the whitefish of which 24,000,000 pounds with a marketed value of over $6,000,000 are taken annually. Next in importance is yellow walleye of which 17,000,000 pounds with a marketed value of $4,000,000 is taken annually. Thi in numerical importance, though only seventh in importance by value is cisco, which name is used to include tullibee, lake herring, chubs and similar fish. Fourth in numercial importance, is blue walleye which is produced almost exclusively in Lake Erie. It is only on the basis of long-ter averages that this species is fourth; for the past two years production has been very low. Other species in order of quantities produced are, pike, perch, lake trout, sturge sauger, carp and catfish. This is not a complete list, but does include all species of which 1,000,000 pounds or more are produced annually.

A variety of fishing gear is used by the
mercial fishermen. The most commonly used type is
gill net, which is made of threads so fine that the
h do not readily perceive it is in the water, and
which they become entangled. Weights along the
tom of the gill net hold it down, generally on the
e bottom, and floats along the top keep the net
rtical. If the lake is ice free, gill nets are
nerally payed out from a boat in a long line, left
er night or longer, then pulled aboard, fish and all.
e fish are then disentangled, and the nets reset. Gill
ts are also set under the ice. Holes are cut in the
e and a rope is passed from one hole to the next by
rious means, then used to pull the gill net into place.

Fish are also caught in various devices which are
signed to lead them through narrow openings, into
closures of heavy netting from which they cannot readily
t out again. Three variations of this type of net
ich are used in fresh waters are the pound net, the trap
t and the fyke net.

In a few places, beach seines are used. A beach
ine is set out parallel to shore, then long ropes which
e attached to either end are slowly drawn ashore until
e fish are completely enclosed between the beach and the
ine.

Very recently, several fishermen on Lake Erie have started to use otter trawls. Otter trawls are dragged along the bottom where they engulf fish. The action of water on the otter boards causes them to pull sideways on either side of the net, which holds it open horizontally while floats on the top of the leading edge of the net hold it open vertically. After being dragged for approximately one hour, the trawl is taken aboard and the fish are poured out on deck.

A major part of our commercial catch of fresh-water fish is sold in the United States. Chicago, Detroit and New York are the main markets with which Canadian producers deal. About three-quarters of the catch are sold as "fresh fish" - that is they reach the wholesaler packed in ice, and otherwise unprocessed, except that the viscera may be removed. The remainder are sold frozen. About half of the frozen fish are sold whole or eviscerated only, the rest are sold as frozen fillets. A very small proportion of the catch, less than 1%, are sold in other forms such as, smoked fish or as canned fish.

An outstanding feature is the extent to which fresh-water fish are <u>not</u> utilized. There are hundreds of lakes in northern Canada that are exploited only lightly, or not at all because inadequate transportation makes it impossibl to fish them. Furthermore, in many cases, legal restrictio prevent lakes from being exploited to their full capacity.

Finally, the price received for certain species
o low that their actual production is small compared
potential production. If there were sufficient
ntive for taking ciscoes, particularly the ciscoes
ne Prairie Provinces which are generally known as
ibees, probably the Canadian production of fresh-water
would be at least tripled. We use only a small part
ur potential production of the various species of
ers, of burbot and of smelt. We make no use of species
as alewives, gizzard shad and of the many species of
ows. All in all, we are using only a small part of our
h-water fisheries resource, and under present conditions,
not likely to do so. However, as the world's population
particularly Canada's population increases, we will
ainly utilize a larger proportion of the resource. The
ein-hungry countries of the world who are looking to
sea for more food are rapidly increasing their fishing
ntial. They will indubitably fish much more heavily
hose parts of the high seas which Canada has always
rded as her fishing grounds. This in turn will inevitably
ease the importance of our fresh-water fisheries.

Increased utilization depends partly on increased
nd for the product. There is a steadily increasing
nd in North America for food packaged in a ready-to-cook
. To sell more fish the fishing industry should take
advantage of this trend to put on the market a product,

not necessarily distinguishable as fish, that is artificially flavoured and ready for the pan. Technologists of the Fisheries Research Board have developed at least two items, fish flour and fish sausage, which could be the starting point for developing such a product. The objective should be a product in which taste and texture are so disguised that the species of fish used would be immaterial.

Increased utilization would also be facilitated by the development of new types of fishing gear. As a step in that direction, the Fisheries Research Board is co-operating with other agencies in an experiment with a midwater trawl in Lake Erie as a means of catching smelt which cannot be efficiently exploited with the gear now available. The Board will also participate in experiments with purse seines and Danish Seines for the same purpose.

Our commercial fishermen are, and always have been, essentially hunters of wild fish. We do not attempt to rear domesticated fish as for market, but it seems very likely that we will develop the utilization of fish in this way as a commercial venture.

ON THE MORPHOLOGY AND LIFE HISTORY OF PHOCANEMA DECIPIENS (KRABBE, 1878) MYERS, 1959 (NEMATODA:ANISAKIDAE)[1]

B. J. Myers

Abstract

Phocanema (synonyms: *Porrocaecum, Terranova*) *decipiens* is described in detail and its probable life cycle outlined. Eggs deposited in salt water develop and hatch in 7 to 14 days at between 10° C and 24° C, even after previous freezing. Temperatures over 24°C are lethal. Larvae fed to a large variety of invertebrates passed quickly through their intestines still alive; fed to fish, they disappeared within 24 hours but in one case a larva was found ensheathed in the intestine. No larvae were found in 'wild' invertebrates although many were infected with free-living nematodes. It is concluded that, while numerous invertebrates may act as 'transport' hosts for the larva to a fish, none acts as a true intermediate host. While larvae infective to seals occur commonly in the muscles of cod, a large variety of other fish are also infected and are a more probable source of infection. Development to maturity in the seal takes approximately three weeks, and it is probable that the main source of the infection in the Gulf of St. Lawrence is the harp seal, although harbor and grey seals also contribute to it.

Introduction

Phocanema decipiens (Krabbe, 1878) Myers, 1959,[2] a member of the family Anisakidae Skrjabin and Karokhin, 1946, subfamily Anisakinae Railliet and Henry, 1912, occurs as an adult in the stomach of numerous species of seals. Because it is a common parasite of grey, harp, and harbor seals in waters off the eastern Canadian coast a study has been made of its morphology and life history.

Adult Morphology

There are three lips with dentigerous ridges; interlabia are absent. Each lip is marked by a median indentation giving it a bilobed appearance. The dorsal lip is constricted at its base and bears two papillae just above the constriction, while the two subventral lips have a single medially placed papilla. The cuticle is conspicuously striated. Cervical alae are absent. The nerve ring and the rounded cervical papillae lie at the same level. The oesophagus is composed of an anterior preventricular region and a posterior ventricular region. An intestinal caecum arising from the intestine extends anteriorly along the oesophagus. A long ribbon-like gland is located on the left side of the anterior third of the body and opens to the exterior by a pore at the base of the subventral lips. Adult males measure from 25 to 70 mm in length and 1 to 1.5 mm in width. The tail is bent ventrally and terminates in a small spined process. Caudal alae are present. There are 50 to 100 pairs

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[2]Synonyms: *Porrocaecum, Terranova*.

of preanal and 6 pairs of postanal pedunculate papillae; the third pair of postanal papillae is the largest and has a double ending. A single sessile, medial, subterminal papilla is present and a pair of phasmids lie posterior to the last pair of postanal papillae. Three dentate ridges are seen posterior to the cloacal opening. The spicules are nearly equal, 1.5 to 2.5 mm in length, and terminate in a hook. The adult females measure from 35 to 80 mm in length and 1.5 to 2 mm in width. The vulva is situated in the middle third of the body. The eggs are spherical, 48 to 56 μ in diameter.

Cuticle and Hypodermis

The body is covered with a non-cellular cuticle beneath which lies the thin hypodermal layer, which is differentiated into four longitudinal chords—two lateral, a dorsal, and ventral—which lie between the muscle cells; posteriorly the dorsal and ventral chords are poorly developed and tend to disappear. When examined in cross section the cuticle is seen to be composed of an external, an internal cortical, and an oblique fiber layer, the internal matrix, and a basal lamella. These layers are the same as those described by von Linstow (5). The coelomocyte cells are found in the anterior region of the body and appear as a chain of independent cells. Branched cells, as described for *Contracaecum*, were not observed.

Musculature

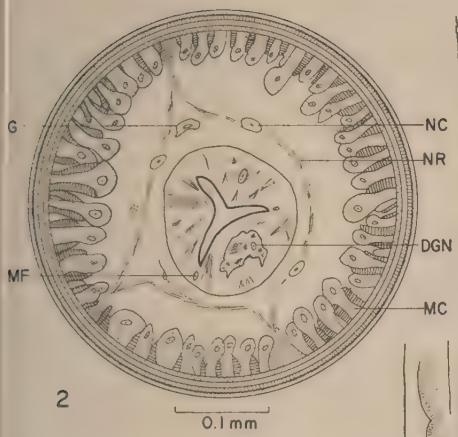
The individual coelomyarian muscle cells are rhomboidal in shape and composed of a fibrillar zone extending up the sides of the cell, and a protoplasmic zone containing the nucleus. Somato-oesophageal muscle extends from the oesophagus to the body wall and somato-intestinal muscles from the intestine to the body wall. The depressor ani muscle is H-shaped and extends between the dorsal wall of the rectum and anus and the dorsal lateral side of the body. A rector spiculi muscle extends from the head of the spicule to the lateral chord and the protractor spiculi from the spicule to the ventral side of the body.

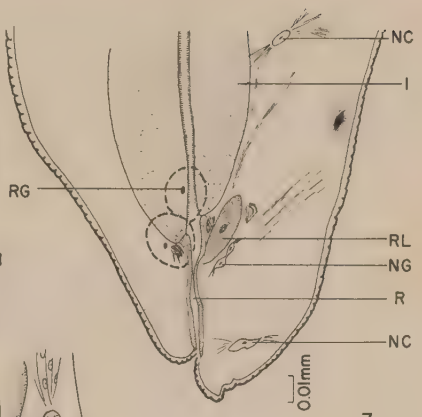
Excretory System

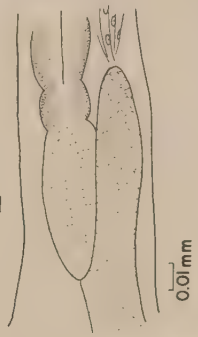
The asymmetrical system consists of a terminal duct and gland with a lateral canal in the left chord. Histological studies show the excretory pore opening between subventral lips as a slit, which connects to a duct the walls of which show cellular divisions. In cross section this wall appears as a layer of dark-staining material. Since the covering of the excretory gland is a syncytial cytoplasm devoid of nuclei, the cells may be epidermal cells which have become invaginated to form a lining for the duct. The ribbon-like excretory gland lies in the perienteric region of the left side of the anterior third of the body. The lumen widens to form an excretory sinus in which there is a single nucleus. Many fine branches connect with the lateral chord. Posteriorly, the gland attaches to the left lateral chord.

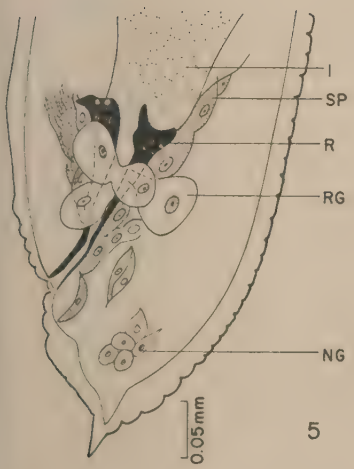
Nervous System

The nervous system consists of a circumoral fibrous ring and ganglion surrounding the oesophagus. Associated with the nerve ring are lateral and









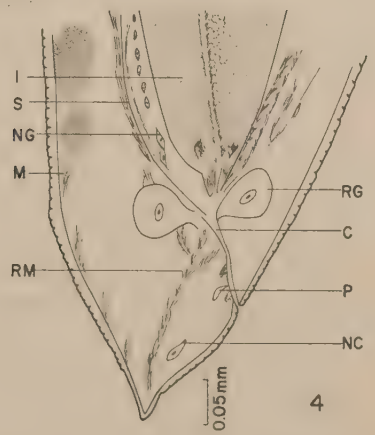


FIG. 1. Oesophageal region. FIG. 2. Cross section of oesophageal region. FIG. 3. Longitudinal section, female tail. FIG. 4. Longitudinal section, male tail (adult). FIG. 5. Longitudinal section, male tail (developing adult).

ABBREVIATIONS:

C, cloaca
DGN, dorsal gland nuclei
EG, excretory gland
I, intestine
M, muscle
NC, nerve cell
NG, nerve ganglion
NMF, nuclei marginal fiber
NR, nerve ring
P, papillae
R, rectum
RG, rectal gland
RL, rectal ligament
S, spicule
SP, spicular pouch

ventral ganglia and the papillary nerves which extend forward to the papillae. Posteriorly, there is a middorsal nerve which arises from the lateral ganglion. A branch from this nerve extends forward to supply the cervical papillae. A detailed study of the posterior and anterior nervous system was not attempted.

Digestive System

Oesophagus

The oesophagus, which appears to be divided into two portions, has a triradiate lumen, one ray of which is directed ventrally, the other two dorsally. The anterior preventricular region extends posteriorly from the lips to the ventil muscles and the ventriculus extends from the ventil muscles to the intestine. Anteriorly the ventriculus becomes dilated to form a bulbular portion. Although Cobb (1) considered this bulbular portion to be a separate region, it is not histologically different from the ventriculus proper. There are radial and marginal oesophageal muscle fibers: the former are numerous, running from the lumen to the sides, and the latter originate at the arms of the triradiate lumen. A ramified dorsal gland arises in the ventriculus, narrows at the level of the preventriculus, and extends forward to approximately the level of the nerve ring, where it opens as a narrow slit into the lumen of the oesophagus. The nucleus of the dorsal gland is curved and lies in the posterior portion of the gland. A subventral gland and two nuclei are observed in the ventriculus. The presence of the two nuclei supports Hsü's (2) observation although Jägerskiold (4) thought the nuclei were degenerate. The oesophagus communicates with the intestine by an oesophageal–intestinal valve.

Intestine

The intestine is an epithelial-lined tube with an outer tunica propria covering. In the ventricular region (Fig. 1) the intestine gives rise to an anteriorly projecting intestinal caecum. The caecum in cross section appears to be composed of "palmate" structures without definite cellular boundaries, arranged radially to form an irregular lumen. The caecum is supported by fibers which terminate in or near the lateral chord. The function of the caecum is unknown. In the female the intestine opens ventrally to the anus. In the male the reproductive system joins the rectum to form a cloaca. The cuticularly lined rectum has three large rectal glands in the female and six to eight in the male. An anal sphincter is present.

Reproductive System

The reproductive system is of the telogonic type, that is, the germ cells proliferate in the proximal portion of the gonad—the germinal zone—which is succeeded by a growth zone where the germinal cells enlarge and become differentiated into gametes.

Male

There is a single testis, the proximal portion of which is covered by a cap cell of epithelial tissue. The spermatogonia form a solid column and as they increase in size they become arranged radially around a rachis. The seminal

esicle is a thin-walled sac in which the epithelial cells are squamous in the nterior portion and columnar in the posterior portion. Posteriorly the seminal esicle narrows to form a canal, opening into a dilated glandular vas deferens hich terminally becomes a chitinous walled rectum opening ventrally to the cloaca. The posterior part of the anal sphincter muscle surrounds the jaculatory duct. Three large ovoid rectal glands are present connected with broad band-like rectal ligament.

Two subequal spicules each terminate in two phalanges. The proximal nd of each spicule is semilunar in shape. Each spicule contains a proto- lasmic core and is surrounded by a cuticle which is continuous with the uticular lining of the spicular canal. The outer layer appears colorless and is ose-fitting, whereas the inner layer is darker and well defined. No nuclei ere observed within the protoplasmic core. Two sets of muscles control the picules: the retractor muscles, which attach to the proximal end of the spicules nd the protractor muscles, which insert posteriorly to the body wall. The picules lie in a spicular pouch. Posteriorly, at the level of the rectal ligament, he spicules enter a chitinized spicular canal; the two canals at first are sepa- ate but unite to form a common canal which opens into the cloaca as a dorsal lit. A gubernaculum is absent.

Female

The ovaries are tubular sacs covered by a simple multinucleated epithelium, hich is very thin in the terminal region. The blind end of the sac is covered y an apical cell. The germinal zone is represented by many small cells crowded o closely together as to make their boundaries difficult to distinguish. These ells divide mitotically to produce oögonia, and a rachis is formed. The achis appears as a central strand of non-nucleated tissue extending to the eginning of the oviducts. The oögonia become "tear-shaped" and are arranged adially around the rachis. The ovaries open into narrow, non-muscular ubular oviducts lined with columnar epithelium. Near the terminal portion f the ovaries the oögonia drop from the rachis and become tightly packed n the oviducts where they assume the typical round shape. There is no lefinite seminal receptacle, the region where the uteri and the oviducts unite erves this purpose. In this region oblique and circular muscles are observed. The seminal receptacle may be occluded in older females. Epithelial cells f this region have become long and form a plug almost closing the uterus roper. Sperm cells can only be observed when ova are absent.

The uteri are lined with squamous epithelial cells. The size and shape of the teri is dependent upon their condition; when they are filled with eggs they ecome distended and when empty, completely compressed. Distally the teri enter a common muscularized tubular vagina. The columnar epithelial ells of the uteri are continuous with those of the vagina but sometimes, due o contraction, appear to be cuboidal. These cells form an irregular lumen hich is usually narrow, allowing the passage of only a single egg. An anastom- sing circular and oblique muscle layer surrounds the vagina, and a connective issue membrane serves as an external covering. The vagina is divided into

two portions: a vagina uteri which is the portion preceding the uterus, and a vagina vera which has a cuticular-lined lumen formed by the cuticle of the vulva. The cells of the vagina vera are larger and more numerous than those of the vagina uteri.

The Egg

The egg is oval in shape and varies in size from 48 to 54μ in diameter. The external covering is smooth and possesses adhesive properties. A chitinous membrane (the shell) and a vitelline membrane comprise the egg coverings.

The Life Cycle

The egg is in the morula stage when passed in the faeces of the seal and certain conditions are necessary for its development to a larva. Although it is impossible to duplicate natural conditions in the laboratory, attempts were made to determine the effects of temperature on the development of the egg.

Eggs were obtained by dissecting the vagina and uteri of adult worms collected from seal stomachs and their stage of development varied from the uncleaved to the morula.

The seal spends most of its life in sea water the temperature of which varies both seasonally and geographically. Eggs which are passed in its faeces must, therefore, be able to withstand these variations. In the laboratory it was found that for development and hatching of the egg, the optimum temperature varied between 10° and 24° C. Even when subjected to previous freezing the egg developed and hatched in from 7 to 10 days. These findings confirmed those of Scott (11). Below the minimum level, development was retarded. At 4° C there was no development beyond the morula stage. Temperatures higher than 24° C were lethal.

Short periods of desiccation are lethal to the eggs and moisture is essential for hatching. The moisture content of the faeces is sufficient to maintain water constancy but prolonged drying on seal "hauling out" sites would kill the eggs.

Development and hatching will proceed in closed petri dishes. Hatching will occur in solutions of seal faeces. When treated with dilute formalin, potassium bichromate, or artificial digestive juices eggs will neither develop nor hatch.

The Larva

Within 7 to 14 days a fully formed, ensheathed larva is observed moving spasmodically within its flexible, transparent, very thin-walled shell. By repeated pushing—produced by coiling and uncoiling itself—the larva succeeds in breaking the egg shell medially and emerges head first. Still adhering by its tail to its castoff shell it thrashes about in the water. If freed from the shell, it will move independently but the thin, flexible tail will adhere to particles of debris and the movement in the water is limited to a thrashing one radiating from the attachment point of the tail. At 4° C the living larvae are seen in a coiled position; raising the temperature produces activation and the movement becomes typical.

Newly hatched larvae, 200 μ long, having molted once within the egg are in the second stage but still enclosed within the cast cuticle. The cuticle is very loose-fitting and at times—especially upon the death of the larva—appears to be lacking. A boring tooth is present. Cephalic structures are not fully formed and a straight, undifferentiated digestive tract is seen. Larvae sink to the bottom of the culture dishes where they remain. Although they have lived for 14 days in no case had the cuticle been shed or further development taken place.

Larvae will live for 11 days (pH 7.2 to 7.5) in a dish in which cod muscle has been placed, for 5 to 7 days in stagnant cultures (that is, with the water unchanged), for 10 to 14 days in sea water, and for 2 days in seal faeces. Placement in artificial digestive juices, freezing, and drying killed the larvae. At 4° C larvae will remain alive although inactive for 30 days.

In order to become sexually mature and complete their life cycle, larvae must return to the seal. Schiffman (personal communication) and Scott (10) have shown that a stage infective to seals occurs in the musculature of a variety of marine fishes.

The remaining gap in the life cycle was the method whereby the second-stage larva from the egg reached the fish. It appeared that either a specific intermediate host—in which some essential development took place—or a merely passive carrier host would be required.

Eggs deposited in seal faeces in the sea are, as has already been mentioned, subjected to various environmental conditions and may pass through pelagic and benthonic zones. Accordingly, all invertebrates living in these zones could be considered as potential hosts—either intermediate or carrier.

Once in the sea, depending upon the degree of action of waves, currents, and density, the faeces will disintegrate and the eggs be deposited at various depths and, unless consumed on the way, will eventually reach the bottom.

If the eggs are consumed by invertebrates in the pelagic zone, their rapid metabolism could allow the eggs to pass through with little or no change. The body temperature of the invertebrate has the same range as its environment. An egg in the morula stage requires 7 days to embryonate at 24° C, 14 days at 14° C. Accordingly, if a pelagic invertebrate is the intermediate or carrier host it is probable that the larva rather than the egg is the infective stage.

If, on the other hand, the eggs reach the bottom and if the temperature of the environment is suitable for development, the eggs will hatch and the larvae may:

(1) be consumed by a filter feed (invertebrate),

(2) be consumed by a scavenger (invertebrate or vertebrate),

(3) be consumed by an invertebrate or vertebrate which is itself consumed by a predator, or

(4) be consumed by a fish.

If the temperature is unfavorable for development the egg may remain in the morula stage until the temperature is favorable.

Field Studies

During the summers of 1953 to 1956, inclusive, investigations were conducted at the Fisheries Research Board of Canada Biological Station, St. Andrews, N.B.; the Marine Biological Station, Grande Rivière; and the Marine Biological Laboratory, Grindstone, Magdalen Islands, of the Quebec Department of Fisheries.

At sea, small beam trawls, small bottom dredges, and other modified gear were used to collect material. In the intertidal, tidal, and littoral zones material was collected by hand, with nets, and with seines. Surface and bottom temperatures were recorded.

Specimens to be used for experimental infections were transferred to aquaria equipped with a continuous flow of salt water, and aerated. With experience, it was possible to learn the methods necessary to keep the various invertebrates alive under laboratory conditions. Their feeding habits, where possible, were studied and experimental infections adjusted to these. Following infection with *Phocanema decipiens* larvae the invertebrates were held for 3 weeks, then dissected under a dissecting microscope and the various parts of the body studied under a compound microscope. The material was then washed from the slides into artificial digestive juices and the residue examined for the presence of larvae.

As many free-living nematodes resemble the hatched larvae of *P. decipiens* contamination with these was avoided by washing each invertebrate in distilled water before examination.

Table I records the invertebrates used for experimental infections. Controls were maintained in each experiment. Larvae survived in these invertebrates (except *Artemia salina*) and in amphipods, mysids, isopods, and fish as second-stage larvae for not more than 24 hours. Frequently, the larvae were passed alive in the faeces within 30 minutes of infection. The infection in amphipods and mysids has been transferred to fish but the larvae disappeared from the intestine of the fish within 24 hours. In a sculpin, however, still ensheathed living larvae were seen although there was no evidence of development.

Many small free-living nematodes living commensally in *Mysis mixta*, *M. stenolepsis*, *Crago septemospinosus*, *Pandulus borealis*, and *P. montagui* were collected; when first observed under the microscope these resembled the hatched larvae of *P. decipiens*. Closer examination showed them to be free-living nematodes which Steiner (12) described as a new species, *Monhystera cameroni*, a commensal of the above-mentioned hosts.

The Infective Larval Stage in Fish

Phocanema decipiens larvae were collected from witch (*Glyptocephalus cynoglossus* (Linnaeus, 1758)); from plaice (*Hippoglossoides platessoides* (Fabricius, 1780)) from North Bay, N.S.; and from cod (*Gadus callarias* Linnaeus, 1758) from Louisbourg, N.S., and Grande Rivière, Que. These larvae are relatively large and are in the stage infective to seals (10).

TABLE I

Phocanema decipiens infections

	Infections	
	Natural	Experimental
	No. specimens examined	
nnelids		
Nereis sp. (sandworms)	75	25
Sea mouse	20	3
rthropoda		
Amphipods		
Anonyx nugax	10	—
Ampelisca sp.	80	50
Gammarus sp.	250	275
Onosimus plautus	6	—
Rachotropis aculeata	15	5
Syrrhoe crenulata	7	—
Cumacea sp.	20	10
sopods		
Iodothea sp.	150	65
lysids		
Mysis mixta Lilljebord	100	100
M. reticula Loven	50	25
M. stenolepsis Smith	50	50
Mysis sp.	250	35
hrimps		
Argis sp.	25	25
Crago septemspinosus Say	150	300
Pandulus borealis Kröyer	25	10
Pandulus sp.	75	85
Spirontocaris groenlandica (Fabricius)	150	300
rabs		
Cancer irroratus Say	30	40
Pagurus kroyeri Stimpson	25	26
obsters		
Homarus americanus	5	10
lollusca		
Buccinum sp. (snail)	20	20
Littorina sp. (snail)	20	30
Mya areinaria L. (clam)	15	14
Mytilus edulis (blue mussel)	25	40
chinodermata		
Starfish	25	30
Sea cucumber	20	20
Sea urchin	30	30
eleosteii		
Pseudopleuronectes americanus (Walbaum) (flounder)	10	10
Gasterosteus aculeatus Linnaeus, 1758 (stickleback)	7	7
Osmerus mordax (Mitchell, 1815) (smelt)	2	2
Lebastis reticulatus (Peter) (guppy)	2	10
Myoxocephalus sp. (sculpin)	5	5

The larvae, which are yellow in contrast to the white of the fish fillet, are o be seen coiled or uncoiled in the fish muscle. Definite encystment of the arvae has not been seen and they appear to move freely within the muscle, articularly if heat is applied. Hard brown cysts containing unidentifiable aseous material are often found within the fish muscle; these may be due to a

host reaction on the death of *P. decipiens* larvae or to the larval stage of the cestode *Grillotia erinaceus* (van Beneden) Dollfus, 1942, which has been described as causing such cysts.

The larva has three bilobed lips, a dorsal one with two papillae and two subventral, each with a single median papilla. Dentigerous ridges are not developed. A characteristic boring tooth is situated near the ventral margin of the dorsal lip but does not appear to be derived from the lip pulp. The cuticle is transversely striated. Four longitudinal chords are present. The nervous system is poorly developed and appears to be confined to a fibrous ring surrounding the oesophagus. The body is composed of a fenestrated membrane of nucleated cells. A clear orange to red-colored fluid fills the perienteric region of the living larvae. The oesophagus is divided into an anterior or proventricular region and a posterior or ventricular one. The ventricular portion opens directly into the intestine. A caecum arises from the intestine and extends anteriorly along the lateral margins of the oesophagus and is attached to the body wall by a ligament. Posteriorly, the intestine ends in a cuticularly lined rectum to which are attached rectal cells. The excretory or ventral gland consists of a single ribbon-like gland lying in the perienteric region ventral to the intestine. This gland is composed of spongy cytoplasm and two canals, one anterior and the other attached to the left lateral chord posteriorly. The anterior canal extends forward and opens as a slit between the two subventral lips. The opening appears to be guarded by a small finger-like projection. The genital primordial is visible as a group of small rudimentary cells. The sexes cannot be distinguished.

The presence of larvae in the muscles of fish is governed by the distribution of the fish and by its diet. The cod, which is commonly infected, is non-selective in its feeding habits. When able it will feed exclusively upon schools of smaller fish but if these are not available it will feed on invertebrates that may be present. Inshore cod tend to be more heavily infected than offshore cod and this fact is correlated with the distribution of seals and to the higher water temperatures which favor development and hatching of the nematode eggs. In summarizing the movement of cod in eastern Canadian waters Mackenzie (6) stated:

> "Movement of the cod was related to submarine physiography, deep channels acting as barriers. Within the area as a whole, movement increased from west to east as does the seasonal range of water temperature. Larger cod moved on an average more than the smaller. Average size of cod increased with depth. Cod of the same size from deeper water moved more than those from shallower water. Movements have been related to spawning in some instances. No large scale well defined movement related to feeding was shown, although concentrations of food are believed to influence movement. Seasonal changes in local fisheries for cod were related to movement indicated by tagging."

The movement of cod from the Nova Scotia region into the Gulf of St. Lawrence and the consequent mixing of cod populations each with a certain

ndex of infection make it difficult to establish all-over incidence figures for the Gulf. Because seals are not selective in their food habits and cod is not a major tem in their diet, it follows that other species of fish must frequently carry he infection to them.

Templeman, Squires, and Flemming (13) have studied the infection in ther fish in Newfoundland. They found that on a weight basis smelt are eavily infected although on an individual basis, the infection seems light. Smelt are inshore fish, prefer a high surface temperature, show little movement except that needed for spawning, and feed upon crustacea, small fish, nd polychaete worms. Tomcod which share similar habits with the smelt re very lightly infected. Haddock are lightly infected but as they live offshore n deeper water perhaps have less opportunity of becoming infected. Pollock lso are lightly infected and feed mainly on pelagic fauna. These authors ound witch, yellowtail, and winter flounder -which consume very few fish— to be lightly infected whereas the American plaice—a fish feeder—is heavily infected, even although the latter occur in shallow water. Herring, capelin, nd other pelagic fish were found to be free of infection; longhorned sculpin, ngler, Greenland halibut, Greenland cod, pollock, yellowtail, sea raven, and red fish were infected.

Small encysted *Phocanema*-type larvae were collected by the author from the stomach wall of skates (*Raja* sp.) from the Magdalen Islands, Que. These larvae had small, developing intestinal caeca and differed from the larvae collected from fish musculature mainly in size.

There are various *Ascaris*-type nematodes in marine animals in the Gulf of St. Lawrence, and *P. decipiens* can be distinguished from others mainly by the presence of an intestinal caecum. However, there are others, particularly *Anisakis*, in which there is no caecum. In all genera, when the young larvae hatch from the egg there is no visible intestinal caecum and as a consequence, except in controlled experiments, Phocanema-type larvae can be recognized with certainty only after development of the caecum and it is unknown when this development takes place. Very small forms without a caecum may belong to any genus and are referred to as *Anisakis*-type larvae. The reproductive and nervous systems are the last to develop whereas increase in size and growth of the digestive tract and excretory systems occur earlier. The distinctive cephalic structures are formed only after the final molt in the seal stomach and consequently recognition of the early larval stages must be based on size and the morphology of the digestive tract.

The larval stages of the Anisakinae have been reported from cod and various ground fish in North American and other regions. These larvae were recognized as early as 1767 and approximately 85 larval forms occurring in fish have been reported, described, and assigned scientific names. It is impossible to attempt to recognize these larval stages on the basis of early descriptions and they must be regarded as *nomina dubia*.

In the structure of the digestive tract larval stages of Anisakinae found in fish and invertebrates resemble the adult stages but the cephalic structures

are only evidenced by the presence of three lips. As many genera possess the same type of digestive tract it is difficult if not impossible to assign the larval stages to genera. Accordingly, I propose the following names, based on digestive tract formation, for the larval stages of the Anisakinae:

(1) Phocanema type: ventriculus present. Anteriorly projecting intestinal caeca present.
(2) Anisakis type: ventriculus present. Anteriorly projecting intestinal caeca absent.
(3) Contracaecum type: ventriculus and ventricular appendix present. Anteriorly projecting intestinal caeca present.
(4) Raphidascaris type: ventriculus and ventricular appendix present. Anteriorly projecting caeca absent.

Both Phocanema-type and Anisakis-type larvae were found in the length class of 17 to 34 mm. Templeman, Squires, and Flemming (13) reported no Anisakis-type larvae longer than 34 mm. It is possible that the young forms lacking a caecum were Phocanema-type larvae in which the intestinal caecum had not yet developed.

The Adult Stage in Seals

The final larval stage, after an unknown number of molts in the stomach of the seal, emerges as a sexually mature adult in 21 days. The general health of a seal does not seem to be affected by massive infections.

As it is impossible to examine seals immediately after death one can only speculate as to the worm's typical method of attachment or its exact position in the body. Adults appear to be free in the lumen of the stomach and intestine although some have been observed attached to the stomach mucosa and to the intestinal wall.

After the death of the seal, worms have been seen escaping through the nostrils, mouth, and rectum. Due to the methods of capturing seals, it is usually 24 hours or longer before examination is possible so that it is impossible to determine accurately what worm population a seal harbored prior to its death. Moreover, the larvae and developing stages are identified with great difficulty since the cephalic structures are not formed at this time.

Anisakid nematodes in seals cannot be identified with accuracy superficially (that is, by gross morphology), nor, in the case of mixed infections, can the percentage of the various genera be estimated. It is necessary to expose the digestive tract of adult worms and to prepare *en face* views of heads of specimens possessing a Contracaecum-type digestive tract. As *Contracaecum* and *Phocascaris* possess the same type of digestive tract they can be separated only on the basis of cephalic structures. Furthermore, many fish upon which seals feed are infected with species of anisakid nematodes which may be transitory parasites in the seal. Identification of adults must be based on specific measurements and morphology.

All seals of the north Atlantic coast of Canada are potential hosts. The harbor seal—the numbers of which are estimated to be 15,000 (3)—is the

most widely distributed resident seal, being found throughout the year from the Bay of Fundy to the north shore of the Gulf of St. Lawrence. These seals are generally considered to be non-migratory although Fisher and Scott (2) observed local migrations in the Bras d'Or Lakes.

The grey seal is more locally distributed. It is estimated (3) that about 7000 occur in Canadian waters. Their principal area of concentration is in the southwestern area of the gulf, with a smaller herd of about 2000 at Amet Island in the Northumberland Straits, and another, of about 400, at Deadman Island, near the Magdalen Islands. Smaller concentrations also occur around Anticosti Island; at Grand Manan, Bay of Fundy; on the northeastern coast of Nova Scotia; in the Bras d'Or Lakes; and at Sable Island.

A population of harp seals (3), variously estimated to be between one and two million, enters the Gulf of St. Lawrence by the Strait of Belle Isle in January and, after whelping and mating, leaves again by the same Strait in May or June. From the limited data available (3) it is considered that they enter the Gulf apparently uninfected with *P. decipiens* but are infected when they leave.

Even although lightly infected the enormous number of harp seals concentrated in the Gulf must be considered a major factor in the dispersal of the eggs of *Phocanema*.

Conclusions

Larval stages of the various genera of the Anisakinae have been observed in the body cavity, mesenteries, and other organs of vertebrates and invertebrates, and there is a considerable variety of developmental patterns.

Thomas (14) found that the dragonfly nymph and guppy were utilized as intermediate hosts for *Raphidascaris canadensis* of the muskelunge. He also observed that the guppy could be infected directly with the embryonated eggs, the intervention of the nymph being unnecessary.

Thomas (15) experimentally completed the life cycle of *Contracaecum spiculigerum* of cormorants. The eggs hatched into larvae and were infective to guppies; after 3 months the larvae were observed in the mesenteries of the guppies. These larvae were infective to cormorants. However, if the infected fish was consumed by another fish, the larvae would re-encyst in the mesenteries.

Mozgovoi (8) found the earthworm to be the intermediate host for *Porrocaecum crassum* of ducks.

Walton (16) found *Rana* and *Siren* sp. to be the intermediate hosts for *Multicaecum tenicollis*.

Experimental evidence of all stages of the life cycle of *Phocanema* is not yet available but from evidence that is available it would seem that:

1. Eggs passed in seal faeces embryonate and after 10 to 14 days develop into second-stage larvae.
2. The hatched, ensheathed larvae are eaten by an invertebrate in which no development takes place (that is, the invertebrate is a carrier or transport host only).

3. The invertebrate is eaten by a fish in the stomach of which the larvae bore into the stomach wall, encyst, and develop (after ensheathment in the muscles) to the stage infective to seals. (This stage can probably occur in any species of ground fish feeding on the proper invertebrates and in an area in which seals have dispersed the eggs.)
4. The seal is infected by eating the fish infected with the larvae, which reach sexual maturity in the digestive tract of the seal.

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MMENTS ON THE DISTRIBUTION AND MIGRATION BIRDS IN FOXE BASIN, NORTHWEST TERRITORIES

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ING the summers of 1955, 1956 and 1957, the CGMV *Calanus* carried out ery surveys in Foxe Basin (Figure 1), and one or more of her crew was

Figure 1. Map of northern Foxe Basin showing localities mentioned in the text

iciently interested in birds to keep notes of the species observed. The *anus* is a fifty-foot ketch operated by the Fisheries Research Board of Can-

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ada, and it has been working in the Eastern Canadian Arctic every summer since 1947 (Dunbar, 1956). The crew normally consists of between four and six men (a skipper, an engineer, one or two scientists, and one or two student assistants). This paper combines their ornithological observations through three years in Foxe Basin in an attempt to add available knowledge about the distribution and migration of birds in the area.

In September 1955 the *Calanus* was taken from Southampton Island northward along the west coast of Foxe Basin to Turton Bay in Igloolik Island where it was permitted to freeze in for the winter. Two of the crew, Bill Black and Ian McLaren, left the ship to return to Montreal, while Ted Grainger and Skipper Hans Andersen remained on board to continue biological research through the winter. Ornithological observations in Foxe Basin during 1955 therefore covered the fall migration and the winter of 1955-1956.

The following spring John Evans arrived at Igloolik on May 27 to help in the expanding research program, and the remaining members of the 1956 expedition arrived later: Tom Wilson, Dan Perey and John Skidmore. During June and July, Evans and the others made short trips by dog sledge to the floe edge east of Igloolik Island, on foot across the island, and by whaleboat to other islands nearby. On August 4 the *Calanus* left Igloolik, and afterwards traveled to different places in northern Foxe Basin carrying out her summer program. She finally reached Frustration Bay, Rowley Island, on September 17, and was pulled onto land above high-water level a few days later. The entire expedition then left the ship and returned by air to Montreal. Ornithological observations in 1956 therefore covered the spring arrival of migrants at Igloolik Island, and include scattered records of birds in northern Foxe Basin throughout the summer.

On May 18, 1957, Andersen and Ellis flew to Frustration Bay to rejoin the ship. They were joined by Evans on June 29, and by Black and Wilson on July 24. Although the *Calanus* was pushed back into the water, she was unable to leave Frustration Bay owing to unusually dense pack ice all summer, and so had to be pulled back onto land for a second winter on Rowley Island. Wilson and Ellis flew south on August 30, Evans and Black remained at Frustration Bay until September 15, and Andersen a few days later. From mid-May to mid-September one or more of the crew was traveling daily and collecting in the bay or its immediate vicinity. The ornithological observations in this area extended from before the arrival of most migrants until after many had departed.

We have been fortunate in having the opportunity to refer to two sets of manuscript notes on birds in Foxe Basin. One was compiled by Tom Manning and Andrew MacPherson as a report to the Department of Mines and Technical Surveys, Ottawa, after the exploration cruise of the CGMV *Nauja* in 1949. The *Nauja* entered Foxe Basin from the southeast in August, took an anticlockwise course around the basin and left through the southwest in September, having stayed at the following localities en route: SW Prince Charles Island (August 16-18), NW Prince Charles Island (August 20-22),

NW Air Force Island (August 23-27), Piling (August 29-September 3), North Spicer Island (September 6), Rowley Island (September 8), Near Ignerit (September 9-11), Cape Jermain (September 17), and Barrow River (September 18). Observations were made at all these localities and at sea in between; the period of these observations coincides with the beginning of the fall migrations. The other set of notes from Foxe Basin was compiled by the scientists and officers on board HMCS *Labrador* during her 1955 and 1956 expeditions to the Canadian Arctic. A preliminary report of the expeditions has been prepared by N. J. Campbell and A. E. Collin as manuscript report No. 13 of the Fisheries Research Board of Canada.

Distribution and breeding records from the *Calanus* and *Nauja* expeditions are summarized in Table 1.

Northern Foxe Basin has hardly been investigated by professional ornithologists, although birds from the region have been described occasionally. Reports from the area include Parry (1824), Hørring (1937), and Bray (1943), plus a few brief comments in Wynne-Edwards (1952), and Ellis (1956). Manning (1943) has written a brief history of exploration of the Basin. Two recent books, *Arctic Birds of Canada* (Snyder, 1957) and *The Arctic Year* (Freuchen and Salomonsen, 1958), contain much information about the region.

Identifications have been largely based on sight records and so, with the exception of Thayer's Gull (see Table 1), are given to species, not subspecies. Names agree with the checklist of the American Ornithologists' Union (1957). Ice terminology follows Armstrong and Roberts (1956), with the exception of the term *floe edge*, which is used in this paper to mean *the edge of the winter ice bound fast to the shore*. This convenient expression is used throughout the Canadian Arctic, by residents and scientists alike, although it has not been adopted for international usage.

Finally we wish to express our gratitude to the other crew members of the *Calanus*, who have contributed data to this paper, particularly to Ian McLaren for most of the 1955 observations; and also to the other personnel of the Arctic Unit of the Fisheries Research Board of Canada for their co-operation during all phases of this work. We are also indebted to Tom Manning and Andrew MacPherson for bringing to our attention their extensive bird notes from Foxe Basin and permission to publish some of their data, and to Neil Campbell of the Atlantic Oceanographic Group for turning over to the Arctic Unit a report of bird observations from HMCS *Labrador*.

OBSERVATIONS DURING 1955

These observations are of interest in revealing some of the species which remain late in high-arctic regions as winter approaches. From September 19 to October 30 McLaren and Black recorded birds in and around Igloolik Island. At that time of year snow had covered the frozen ground, and ice was beginning to form in the bays and inlets. The birds seen were generally marine forms. A white-winged (Glaucous?) gull was seen on September 20, 4 Guillemots and 6 Dovekies on September 24, another Dovekie on October 4, at least 4 Ivory Gulls on October 7 and several more Ivory Gulls through October until the

TABLE 1. SUMMARY OF DISTRIBUTION AND BREEDING RECORDS DURING CALANUS AND NAUJA EXPEDITIONS TO FOXE BASIN

	Calanus records					*Nauja* records							
	Igloolik Island and vicinity	North Oogjit Island	Mogg Bay	Frustration Bay and Butterfly Lake	East Melville Peninsula 68° 45'N 81° 13'W	SW Prince Charles Island	NW Prince Charles Island	NW Air Force Island	Piling	North Spicer Island	NE Rowley Island	Igerit	Cape Jermain	Barrow River
Common Loon, *Gavia immer* (Brünnich)	–	–	–	–	–	–	–	–	x	–	–	–	–	–
Arctic Loon, *Gavia arctica* (L.)	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Red-throated Loon, *Gavia stellata* (Pontoppidan)	b	–	–	x	–	b	–	–	–	b	–	–	–	–
Brant, *Branta bernicla* (L.)	b	x	–	x	–	b	b	x	x	x	–	–	–	–
Snow Goose, *Chen hyperborea* (Pallas)	b	x	–	–	b	x	x	x	–	–	?	–	–	–
Blue Goose, *Chen caerulescens* (L.)	x	x	–	x	–	–	–	x	x	–	x	–	–	–
Oldsquaw, *Clangula hyemalis* (L.)	–	–	–	x	–	–	–	–	–	–	–	–	–	–
Common Eider, *Somateria mollissima* (L.)	b	b	x	b	x	x	–	x	–	–	x	–	–	x
King Eider, *Somateria spectabilis* (L.)	b	b	–	x	–	–	–	–	–	–	–	x	–	x
Rough-legged Hawk, *Buteo lagopus* (Pontoppidan)	b	x	–	b	?	b	x	x	?	–	?	?	?	–
Gyrfalcon, *Falco rusticolus* L.	–	–	–	–	–	–	–	–	–	–	–	–	–	x
Peregrine Falcon, *Falco peregrinus* Tunstall	x	–	–	x	–	–	–	x	b	–	–	–	–	x
Willow Ptarmigan, *Lagopus lagopus* (L.)	x	–	–	?	–	–	–	–	–	–	–	x	x	x
Rock Ptarmigan, *Lagopus mutus* (Montin)	–	–	–	x	–	–	–	–	–	–	–	–	–	x
Semipalmated Plover, *Charadrius semipalmatus* Bonaparte	–	–	–	x	x	b?	–	–	–	–	–	–	–	–
American Golden Plover, *Pluvialis dominica* (Müller)	–	–	–	x	?	b	x	x	–	–	–	–	–	–
Black-bellied Plover, *Squatarola squatarola* (L.)	b	–	–	x	?	x	–	?	–	–	–	–	–	–
Ruddy Turnstone, *Arenaria interpres* (L.)	b	x	b	x	–	–	–	–	–	–	–	–	–	–
Whimbrel, *Numenius phaeopus* (L.)	–	–	b	x	–	?	–	–	–	–	–	–	–	–
Purple Sandpiper, *Erolia maritima* (Brünnich)	x	–	–	b	–	x	x	x	–	x	–	x	–	–
Pectoral Sandpiper, *Erolia melanotos* (Vieillot)	–	–	–	–	–	x	–	–	–	–	–	–	–	–
White-rumped Sandpiper, *Erolia fuscicollis* (Vieillot)	b	–	–	x	x	x	x	x	–	x	–	–	–	–
Baird's Sandpiper, *Erolia bairdii* (Coues)	x	–	–	pb	x	–	–	–	b	–	–	–	–	–
Dunlin, *Erolia alpina* (L.)	x	–	–	–	–	–	–	–	–	–	–	–	–	–

TABLE 1. (*Continued*)

	Calanus records						*Nauja* records						
	Igloolik Island and vicinity	North Oogjit Island	Mogg Bay	Frustration Bay and Butterfly Lake	East Melville Peninsula 68° 45'N 81° 13'W	SW Prince Charles Island	NW Prince Charles Island	NW Air Force Island	Pilling	North Spicer Island	NE Rowley Island	Ingerit	Cape Jermain	Barrow River
Semipalmated Sandpiper, *Ereunetes pusillus* (L.)	–	–	–	b	–	x	–	–	–	–	–	–	–	–
Hudsonian Godwit, *Limosa haemastica* (L.)	x	–	–	–	–	–	–	–	–	–	–	–	–	–
Red Phalarope, *Phalaropus fulicarius* (L.)	b	x	–	b	?	x	x	x	x	–	x	–	x	–
Pomarine Jaeger, *Stercorarius pomarinus* (Temminck)	x	–	–	x	x	–	–	b	–	–	–	–	–	–
Parasitic Jaeger, *Stercorarius parasiticus* (L.)	b	–	b	x	–	x	x	x	x	x	x	–	–	–
Long-tailed Jaeger, *Stercorarius longicaudus* Vieillot	b	x	b	x	–	x	x	–	x	–	–	–	–	–
Glaucous Gull, *Larus hyperboreus* Gunnerus	?	–	–	x	x	–	x	–	b	–	–	x	–	x
Herring Gull, *Larus argentatus* Pontoppidan	b	?	–	x	?	x	x	x	x	x	x	x	x	–
Thayer's Gull, *Larus argentatus thayeri* Brooks*	?	?	–	?	?	–	–	–	–	–	–	–	–	–
Ivory Gull, *Pagophila eburnea* (Phipps)	x	–	–	–	–	–	–	–	–	–	–	–	–	–
Sabine's Gull, *Xema sabini* (Sabine)	b	b	–	b	x	b	b	–	–	–	–	–	–	–
Arctic Tern, *Sterna paradisaea* Pontoppidan	b	b	–	b	–	b	b	b	–	–	x	–	–	–
Dovekie, *Plautus alle* (L.)	x	–	–	–	–	–	–	–	–	–	–	–	–	–
Black Guillemot, *Cepphus grylle* (L.)	b	b	–	x	–	–	–	–	x	x	x	–	–	–
Snowy Owl, *Nyctea scandiaca* (L.)	–	–	–	–	–	–	–	x	x	–	x	–	–	–
Horned Lark, *Eremophila alpestris* (L.)	x	–	x	x	–	x	–	x	–	–	–	x	–	–
Water Pipit, *Anthus spinoletta* (L.)	–	–	–	–	–	x	x	–	x	–	–	x	–	–
White-crowned Sparrow, *Zonotrichia leucophrys* (Forster)	–	–	–	x	–	–	–	–	–	–	–	–	–	–
Lapland Longspur, *Calcarius lapponicus* (L.)	x	–	–	b	x	x	x	x	x	x	x	x	–	–
Snow bunting, *Plectrophenax nivalis* (L.)	x	x	–	b	x	x	x	x	x	x	x	x	–	–

*Thayer's gull is called *Larus argentatus thayerii* here in agreement with the A.O.U. checklist, although Thayer's gull may be considered a distinct species from the Herring gull. They can usually be distinguished in the field.

x—presence recorded ?—tentative identification b—nests or juveniles recorded.

27th. By that time the sea around the island was covered by surface ice and the nearest open water was at the floe edge to the east of Igloolik Island.

On October 30, McLaren saw a white-phase Gyrfalcon in Igloolik village and this bird, when flying away, disturbed a flock of 7 Snow Buntings.

OBSERVATIONS DURING 1956

The arrival of birds at Igloolik Island

The only birds seen near Igloolik Island during the winter of 1955-1956 were Black Guillemots at the floe edge east of the island. The first spring arrivals were eider ducks (probably King Eiders), which were first seen on April 11 in large flocks at the floe edge and often afterwards. On April 17 Snow Buntings were seen near the *Calanus* but were not noticed again until June 8 at the floe edge. Common Eiders were seen on June 5, and the next day birds were seen in large numbers. There was a flock of about 300 gulls belonging to the Herring - Thayer's Gull complex (see page 66) and many Oldsquaws at the floe edge. A Peregrine Falcon, Baird's Sandpipers, one Dunlin, and Lapland Longspurs were seen on June 8 and Glaucous Gulls tentatively identified. On June 14 groups of Long-tailed Jaegers and a pair of Black-bellied Plovers were seen. Red-throated Loons, Brant, Snow Geese, Ruddy Turstones, Parasitic and Pomarine Jaegers, Sabine's Gulls, and flocks of Arctic Terns were seen on June 18, and Red Phalaropes on June 20.

The spring arrival of birds was therefore spread over several weeks. The eiders arrived early in April, but most other sea birds during the second and third weeks of June. Snow Buntings also arrived about the middle of April, with other land birds almost two months later.

Most sea birds arrive in the arctic regions before the land clears sufficiently of snow to permit them to nest. After their arrival they may spend several weeks swimming and flying along the floe edge, making short flights inland or over the shore-fast ice. Some species, such as King Eiders, Oldsquaws and Arctic Terns, spend much of this period sitting or standing on the ice at the floe edge. The first signs of birds moving inland at Igloolik Island were noticed on June 18 when Evans saw Turnstones and Long-tailed Jaegers. By June 25 and 26 King Eiders and Oldsquaws had started to settle on small pools; pairs of these birds were first noticed on June 29. During the last week of June many different species were seen inland. In addition to the species already mentioned they included Red-throated Loons, Black-bellied Plovers, Red Phalaropes, Herring Gulls, Sabine's Gulls, and Arctic Terns.

North Ooglit Island

Evans traveled to this island on July 4 in an Eskimo whaleboat. At the island's floe edge he saw about 100 Black Guillemots and between 1,000 and 2,000 male King Eiders. Inland on gravel patches he saw a pair of Ruddy Turnstones, about 200 Sabine's Gulls and the same number of Arctic Terns; scattered over the tundra about 30 Common Eiders, 40 Oldsquaws, and 20 Snow Buntings; on ponds 10 Brant, 2 Snow Geese, 2 Red-throated Loons, and 6 Red Phalaropes. He also noticed single Long-tailed Jaegers, and Herring

(or Thayer's) Gulls flying, plus nests of Common Eiders, Oldsquaws, Sabine's Gulls, Arctic Terns, and Black Guillemots (under boulders).

Mogg Bay

Evans visited Mogg Bay by Eskimo whaleboat on July 18 and 19 and saw about 50 Oldsquaws in the mouth of the bay, a single Horned Lark, a single pair of nesting Parasitic Jaegers, Long-tailed Jaegers, and Ruddy Turnstones.

OBSERVATIONS DURING 1957

The arrival of birds at Frustration Bay, Rowley Island

Andersen and Ellis arrived at Frustration Bay on May 19. Some migrant birds had preceded them. There were enormous flocks of large ducks flying along the floe edge on the west coast of Foxe Basin at latitude 68°30'N. Presumably these were King Eiders. In Frustration Bay itself the only signs of birds were a few ptarmigan tracks in the snow. The tracks were probably made by Willow Ptarmigan, which was the only species of ptarmigan seen during the summer.

The next arrivals were Snow Buntings, which were first heard on May 21, and finally seen on May 29. Flocks were seen repeatedly until June 6, but afterwards the buntings occurred only singly, in pairs, or in small groups. On May 29 a large dark bird flew rapidly by the ship and was tentatively identified as a Peregrine Falcon. This early record for a predatory bird is not improbable; many arctic residents maintain that "hawks" are amongst the first spring arrivals.

The next day, May 30, Ellis saw 2 gulls circling over his sledge as he traveled overland by the Butterfly River. Two days later numbers of gulls were seen in different places near the ship. Most of them were either Herring or Thayer's gulls but there were also a few Glaucous Gulls. A single Sabine's Gull was seen on June 3, but no more until June 10.

On June 5 Andersen and Ellis reached the floe edge for the first time and saw King Eiders and Oldsquaws there. Jaegers were first seen on June 7 and 8 but could not be identified to species on those days. On June 10 after 4 days of strong southerly winds, Andersen and Ellis again collected at the floe edge and saw literally thousands of birds. King Eiders and Oldsquaws were most numerous, occurring in countless flocks of up to several hundred birds. The two species were usually separate, but mixed flocks were seen. At least 2 male Common Eiders were seen in one flock of Kings. There were flocks of up to several hundred Red Phalaropes, and smaller, looser groups of Arctic Terns with occasional Sabine's Gulls. Black Guillemots occurred singly or in small groups flying and swimming amongst drifting ice. A single Ruddy Turnstone was also seen.

After the first mass arrival of sea birds on June 10, others appeared in a less spectacular fashion. An unidentified goose was seen on June 12, loons were first seen on June 14, Lapland Longspurs on June 15, White-rumped Sandpipers on June 16, Semipalmated Plovers, Black-bellied Plovers and Purple Sandpipers on June 17. A number of other species were seen later, but June 17 was the last date that probably agreed with the arrival date of any species.

Most of the smaller birds seemed to arrive during the week of June 10-17, shortly after the sea birds appeared at the floe edge.

Frustration Bay

The winter cover of snow and ice in Frustration Bay started to melt early in June; and, as the sea ice began to crack later in the month, large pieces of it broke away from the floe edge. In July the fast ice melted along the shores and the ice cover floated freely in the bay. It was prevented from drifting away immediately by very close pack ice which had pressed into the floe edge in the middle of June. This pack ice and the bay ice remained in Frustration Bay until late August, preventing the *Calanus* from leaving her winter quarters.

Shortly after the spring arrival of sea birds on June 10 the number of species and individuals at the floe edge began to decrease. In particular, the flocks of Red Phalaropes left the floe edge and were afterwards seen only in pairs or small groups inland.

Ducks remained longer at the floe edge. Flocks of Oldsquaws were seen in Frustration Bay until July 17, although many moved inland during late June. Evans and Wilson also saw large flocks, apparently immatures, on August 9 south of the bay. Breeding pairs of this species left the floe edge during June, although returning intermittently to feed, and the flocks seen afterwards were possibly nonbreeding or immature birds.

Pairs of King Eiders were seen inland during late June, but in July there were still flocks of them in Frustration Bay and offshore. In the middle of July these flocks changed their character. Up to that time they had consisted of several hundred birds or less of both sexes, but they were replaced by vast flocks consisting of several thousand males in breeding plumage. The big flocks of males were last seen on July 20 although a few smaller groups were noticed until July 31. Afterwards only small groups of brown eiders (immatures, nonbreeding females or juveniles) were seen. The general pattern of King Eider distribution during the summer therefore seemed to be as follows. Flocks of up to several hundred male and female birds occupied the floe edge during May and June. Pairs of breeding birds moved inland at the end of the latter month. Males returned to the sea in July and assembled in large flocks offshore. They left at the end of the month. Nonbreeding birds and immatures remained offshore throughout August and were joined by juveniles and females towards the end of the month.

A few species of sea birds remained constantly in Frustration Bay throughout the summer. Every day screeching flocks of Arctic Terns could either be seen or heard in the bay, particularly at Needle Point where the turbulent water provided a continuous supply of marine food. Loons were fairly common throughout the summer. A few Red-throated Loons were seen almost every day, but Pacific Loons were rarer.

At least 3 species of gulls occurred in Frustration Bay. Sabine's Gulls were the rarest, being seen only occasionally. The majority of large gulls had a dark mantle with black or brown primaries and belonged to the Herring-Thayer's Gull complex. One adult specimen, which was collected, had glaring

yellow eyelids and was identified as a Herring Gull, while another collected specimen was an immature bird with flesh-colored eyelids; it was tentatively called a Thayer's Gull. Most of the gulls were not identified specifically. They concentrated in late May and early June at the garbage dump, but later around the fish-drying racks near the ship and at the small fishing lake. A pair of these gulls was seen frequently along the lower part of Butterfly River and may have nested there. No other signs of nesting were noticed. A few of the large gulls seen during the summer were identified as Glaucous Gulls.

Black Guillemots were seen at the floe edge only in June and early July. They probably left the vicinity of Frustration Bay shortly after they were last seen there.

Butterfly Lake and vicinity

The arrival of most birds in this region coincided with the spring thaw. Between June 10 and 17 much snow melted and streams began to flow. Land vegetation appeared through the snow cover, and a few days later insects began to appear. Once the snow melted the nature of the land became apparent. There was fertile ground with abundant vegetation around the rivers, but these patches were separated by gravel ridges, which in many places were entirely barren although they generally had a sparse vegetation. Birds usually kept to the valleys but a few were encountered on the ridges. A pair of Semipalmated Sandpipers nested at the side of one ridge running along the west shore of Frustration Bay and terminating at Needle Point. Four eggs were laid between July 11 and 12; three of these hatched on August 2 but the other was deserted.

A valley extended from the head of Frustration Bay to Butterfly Lake and beyond. This valley was regularly walked by the crew of the *Calanus* in order to tend gill nets along the length of Butterfly River. Snow Buntings and Lapland Longspurs were seen regularly in the valley, and also shore birds, including Semipalmated Plovers, Black-bellied Plovers, Ruddy Turnstones, Semipalmated Sandpipers, White-rumped Sandpipers, Purple Sandpipers, and Red Phalaropes. Nests of Snow Buntings and Red Phalaropes were found. The Snow Bunting's nest was first seen on June 28 with 5 eggs, the young hatched on July 11 and left the nest on July 22, although they remained near the nest for some time afterwards. The Red Phalarope's nest was first seen on July 11 with four eggs, and the young were found about 50 yards from the nest on July 24. They were not seen again. Juvenile Purple Sandpipers, Baird's (?) Sandpipers, Lapland Longspurs, and Snow Buntings were seen during the summer, the last two species frequently.

Oldsquaws were seen regularly in the valley early in the summer but only one nest was found. Red-throated Loons and Arctic Terns were common. One young tern was seen flying along the river on August 26. Terns actually were ubiquitous in the vicinity of the *Calanus* and fed from a great variety of habitats: in the sea (three stomachs containing fish larvae), along the Butterfly River (one stomach containing Tipulid larvae), and from fresh-water ponds (no stomachs examined but the ponds were rich with a variety of Entomostraca).

Less regularly seen in the valley were three species of jaegers (Long-tailed Parasitic and Pomarine), Pacific Loons, Sabine's Gulls (one juvenile seen), and Willow Ptarmigan. An unusual record was a small sparrow seen on July and again the next day. It was identified as a White-crowned Sparrow, both by its striped crown and by its song.

A second valley to the west of Butterfly Lake had a similar but more abundant bird population. Ruddy Turnstones, Black-bellied Plovers and Red Phalaropes in particular were far more abundant than in the Butterfly River valley. A single Horned Lark was seen on July 7.

Black and Wilson walked to the east coast of Rowley Island in the same latitude as Frustration Bay on August 20. They saw a flock of about 30 Snow Geese (Lesser?) and two Blue Geese, a group of Sabine's Gulls, and Purple Sandpipers.

The west coast of Foxe Basin, latitude 68° 30' N

Ellis and Wilson were detained four days in this region from August 31 to September 4 while returning to Montreal by aircraft. The coast consists of a series of raised gravel beaches containing many shallow pools and bounded at sea level by stony beaches with mud patches. On August 31 many shore birds were congregated along the coast and the following species were identified: Semipalmated Plovers, Golden or Black-bellied Plovers, Ruddy Turnstones, Baird's Sandpipers, White-rumped Sandpipers, and (Red?) Phalaropes in winter plumage. There were undoubtedly other species present. About 200 gulls, mostly of the Herring - Thayer's Gull complex but also Glaucous Gulls, were gathered around a garbage dump along the shore. A single Sabine's Gull was also seen. A few jaegers (only Pomarine identified), several loons, and small groups of Lapland Longspurs and Snow Buntings were also seen.

About 300 ducks were scattered in an unnamed bay along this coast, in the water, on pieces of ice, and on shore. Most of these were eiders in small groups of 1 or 2 females with juveniles, but there were also several flocks of Oldsquaws. A flock of about 200 Brant and several smaller flocks were disturbed on shore. The large flock, at least, contained both adults and juveniles.

From all appearances the birds observed during this period had left their summer haunts and were assembling along the coast prior to migrating south.

The departure of birds from Frustration Bay

The first sign of departing birds in 1957 was the appearance of flocks of up to a hundred male King Eiders flying up and down the Butterfly River during the period July 6-22. Rowley Island is in the same latitude as Clyde River, Baffin Island, where Wynne-Edwards (1952) saw hundreds of migrating King Eiders flying eastwards between July 14 and August 17, 1950. He postulated that the Clyde River valley was a major migration route for male King Eiders traveling from Arctic Canada to Disco Bay in West Greenland. The departure of males during late July, possibly crossing Rowley Island via the Butterfly River, fits neatly into the pattern of migration suggested by Wynne-Edwards.

The next indication of the fall migration was the appearance in early August of a larger number of shore birds along the Butterfly River than had been seen there during June and July. Small groups of the resident species, which previously had occurred only in scattered pairs, were seen almost daily along the river banks. In addition Golden Plovers were seen regularly, and of course many of the shore birds remained unidentified. On August 13 Ellis walked through the valley west of Butterfly Lake and found it almost deserted. In early August, also, Snow Buntings and Lapland Longspurs began to form small groups along the Butterfly River.

In late August and the first week of September Evans and Black were still able to observe a few birds near Frustration Bay. They included Red-throated and Pacific Loons, plovers (Black-bellied or Golden) and Ruddy Turnstones, unidentified jaegers and gulls (including 1 juvenile Sabine's Gull), Arctic Terns, Lapland Longspurs, and Snow Buntings. A single Gyrfalcon and a Snowy Owl were seen on September 7, the last day that any observations were made.

CONCLUDING COMMENT

Our observations on migration were few but they indicate the periods during which birds arrive and depart in northwest Foxe Basin.

In both 1956 and 1957 two species – King Eiders and Snow Buntings – arrived early in April or May, but most other species during the first two or three weeks of June. In 1957 the sea birds preceded most of the smaller ground birds by a few days.

We have given some information about the southward migrations in both 1955 and 1957. Male King Eiders were the first obvious departures in 1957, leaving during the last week of July. Manning reports seeing only 1 male in Foxe Basin in August and September, 1949, although he recorded hundreds of brown eiders. In August 1957, many species of shore birds and ducks congregated along the shores preparatory to flying south. Manning noticed the same phenomenon in 1949. A number of species were still present in early September in 1957, and Manning reports seeing flocks of many species during the same period in 1949, particularly Lapland Longspurs and Snow Buntings. He also reported seeing flocks of Red Phalaropes at sea between September 4 and 15.

In 1955 near Igloolik most birds had left by the middle of September, even eiders and Oldsquaws which are known to occur there abundantly during the summer and which can remain late in high-arctic regions. Only a few gulls and alcids were seen at sea; all but a few Black Guillemots left in late September or early October. Snow Buntings and Gyrfalcons stayed almost until the last day of October.

The Dovekies and Ivory Gulls that McLaren saw in the fall of 1955 were probably migrating birds and not residents, as there is no evidence that either species nests in Foxe Basin. Fulmars, Ivory Gulls, Dovekies and Brünnich's Murres have been reported occasionally by several different authors in northern Foxe Basin, presumably in migration from breeding colonies in northern Baffin

Island or even farther north. It is interesting that Kittiwakes, which nest in the same general areas as the preceding four species, have not yet been certainly reported in northwest Foxe Basin.

Black Guillemots were the only species of birds recorded near Igloolik during the winter of 1955-1956.

The most distinctive feature of the marine avifauna of northern Foxe Basin, a feature which was noticeable even during our limited time in the country, and which Manning's notes confirm, was the absence of fulmars, kittiwakes, and alcids other than Black Guillemots; in fact, all species which are completely dependent on the presence of cliffs for nesting sites. These species are amongst the commonest birds offshore on the east coast of Baffin Island, and presumably off any arctic coast where they nest. Their absence from northwest Foxe Basin during 1956 and 1957 is apparently typical. Previous authors either do not mention them or state that they occur rarely; nor do the *Labrador* reports mention them in Foxe Basin. There are few extensive cliffs in the basin which could provide these species with suitable colonial nesting sites. The marine species seen in 1956 and 1957 were all able to utilize flat or hilly land for nesting. Even the Black Guillemot can do this. The nature of the coast in Foxe Basin prevents the sea birds, which require extensive cliff nesting sites, from becoming residents.

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HERRING STICKWATER VISCOSITY

Identity of the Gel Factor in Herring Solubles and Means of Overcoming Its Effect

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Herring solubles, prepared by concentrating herring stickwater after acid treatment, varied in viscosity and capacity to gel depending on the season when the herring were caught. Solubles from herring caught in the late fall and winter had a much higher viscosity and tendency to gel than the product from fish caught in the summer and early fall. This change in viscosity correlated with an increase in the gelatin content of the solubles and paralleled the development of milt and roe in the fish. Proteolytic enzyme treatment of stickwater prior to its condensation to solubles was effective in overcoming the action of the gelatin. The capacities of various commercially available proteolytic enzyme preparations to produce solubles with desirable physical characteristics were compared.

HERRING STICKWATER is the water phase of the press juice obtained when whole herring are "reduced" to fish meal. To prepare a fluid concentrate of this by-product, referred to as solubles, suspended and dissolved proteins must first be either removed or destroyed. In one process, the proteins are coagulated by acidulation of the stickwater and then removed by centrifugation (*10*). In another, the protein molecules are reduced to smaller fragments by the action of proteolytic enzymes (*5*).

The acidulation process has been used exclusively in this area of the Pacific coast for some years. Even though the same process was applied to the stickwater throughout the year, the physical properties of the resulting solubles varied with the season. While solubles prepared from summer herring was fluid, that produced from late fall and winter herring was viscous and tended to gel upon standing.

The purpose of this study was to investigate the reasons for the seasonal change in viscosity of herring solubles and to determine how a product with satisfactory physical characteristics could be produced throughout the year.

Experimental

Special Apparatus. The centrifuge used for all pilot plant operations was a Centriwesta Model NWA 205 (Merco Centrifuge Co., San Francisco, Calif.). This centrifuge has a continuous flow arrangement and applies a force of approximately 7500 × gravity. It can be operated with either a chamber or a disk bowl.

Source of Materials. Fresh whole Pacific herring (*Clupea pallasii*) were obtained from local fishing companies and stored at 10° F. until used.

When large volumes of stickwater, representative of a particular season were required, arrangements were made to obtain primary stickwater from a local plant. These samples were held at 10° F. until needed. As a further precaution against bacterial deterioration of the stickwater, Aureomycin was added to all samples as a preservative, before freezing, at a level of 2 p.p.m. (*7*).

A pilot plant was designed to approximate commercial plant conditions as closely as possible (*3*). There whole herring were steam cooked and pressed. The expressed fluid was heated to 212° F. and maintained there for 15 minutes to achieve maximum oil separation. After oil removal by centrifugation, the primary stickwater was treated with either acid or enzyme.

The acid treatment consisted of lowering the pH of the primary stickwater to 5.0 with concentrated sulfuric acid and heating to 170° F. for 15 minutes. The temperature of the stickwater was then raised to 212° F. and centrifuged twice, first with the chamber bowl of the Centriwesta centrifuge to remove coagulated protein and then with the disk bowl to separate oil released by the acid and heat.

For enzyme treatment the temperature and pH of the stickwater were adjusted to the optimum for the enzyme under investigation. Following the addition to the stickwater, of a slurry of enzyme in water, digestion was allowed to proceed for the required length of time. At the completion of each enzyme digestion period the stickwater substrate was boiled for five minutes at a pH of 3.0 to insure enzyme deactivation. The stickwater was then cooled and the pH was readjusted to the desired level.

The treated stickwater was concentrated in vacuo by one of two methods: If a large quantity of solubles was required, the pilot plant concentrator was

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used; for a small volume of solubles, concentration was carried out using a laboratory-size glass evaporator equipped with an antifoam head. Unless otherwise indicated, samples were evaporated to the solids concentration prevailing in commercial solubles, 50 ±1%.

In Table I a comparison is made of pertinent analytical data obtained from solubles produced in the pilot plant and from a product obtained commercially. Both samples of solubles were prepared by the acidulation process from herring caught in the same month. The most pronounced differences between the two products were found in the significantly lower levels of oil and insoluble solids in the pilot plant product. This difference can be attributed principally to the greater efficiency of the pilot plant centrifuge. It was not possible to obtain a centrifuge for use in the pilot plant, scaled down directly from those used commercially. Furthermore, the pilot plant arrangement afforded a finer degree of control of the rate of flow of stickwater into the centrifuge than would be possible under commercial conditions.

Viscosity Measurements. Viscosity was measured by two methods. In the first, a Stormer viscometer (*15*) equipped with a paddle-type rotor driven by a 200-gram load was used. This instrument is used in the local industry for product control work. A soluble is not considered suitable for commerce in this area if the Stormer reading on it exceeds 30 seconds. The instrument, however, can give erroneous information when the product tends to gel (*17*). To overcome this disadvantage a supplementary measurement, the pour residue test, was devised. In this test a 50-ml. beaker containing a weighed amount of solubles was placed in a water bath, at a fixed temperature, for a stated period of time. The beaker containing the sample was then placed at an angle of 45° and allowed to drain for 30 minutes and the percentage by weight remaining in the beaker was determined. The viscosity was expressed as the per cent retained in the beaker under the given conditions.

The viscosity of certain samples did not reach a maximum until some time had elapsed after their preparation. To obtain a measure of this thixotropic effect, both Stormer and pour residue tests were conducted at 2-hour and 24-hour intervals after the solubles had been prepared. Because the viscosity increases as the temperature decreases and it is desirable that solubles remain fluid even in winter, viscosities were measured at 50° F. as well as at room temperature.

Determination of Total Solids. The total solids content of the herring stickwater and solubles was determined by means of a Cenco moisture balance (Central Scientific Co., Chicago, Ill.).

Determination of Insoluble Solids. A Gooch crucible with an acid-washed asbestos mat was prepared and weighed after drying. An 0.5-gram sample of solubles was mixed with 2.0 grams of Celite filter aid suspended in 100 ml. of water and filtered through the crucible with the aid of suction. The filter was then thoroughly washed with successive 30-ml. aliquots of ethyl alcohol, diethyl ether, and acetone. Following this, the crucible was dried and weighed. The amount of solids retained in the crucible, corrected for the amount of Celite added, was referred to as the insoluble solids and expressed as per cent by weight of the original solubles.

Determination of α-Amino Nitrogen. Free α-amino nitrogen was determined by Sahyun's (*16*) modified Sorensen formol titration method.

Extraction and Determination of Gelatin. Approximately 2-gram samples of herring solubles, accurately weighed, were acetone dried, and the gelatin was extracted by the method of Neuman and Logan (*14*). Trichloroacetic acid was added to the extracts to a concentration of 5% (w./v.) and the nongelatin proteins which precipitated were removed by filtration (*8*). The precipitate was washed with 5% trichloroacetic acid, and the filtrate and washings were then combined, evaporated to dryness, dissolved in water, and diluted to 10 ml. Aliquots of this gelatin extract were hydrolyzed in sealed combustion tubes at 230° F. for 6 hours with 6*N* hydrochloric acid, decolorized with charcoal, and filtered. After dilution to volume, hydroxyproline was determined on the filtrates, by the modified method of Martin and Axelrod (*12*), which was further modified to include a heating period of 15 minutes at 80° C. after the addition of ferrous sulfate to ensure the removal of peroxide.

To establish whether hydroxyproline could be determined directly in the hydrolyzates obtained, a known volume of the hydrolyzate was chromatographed on Whatman No. 1 filter paper using water saturated 1-butanol–acetic acid as a solvent (*1*). The spot corresponding to hydroxyproline was eluted and the method described above for the colorimetric determination of the amino acid was applied to the eluate. The results obtained with three samples of solubles showed that the hydroxyproline values obtained by the chromatographic technique differed from those obtained by direct determination on the hydrolyzate by less than 1%.

To obtain a value for hydroxyproline in herring gelatin, gelatin was extracted from fresh herring scales by the method of Burley and Solomons (*2*) and analyzed for hydroxyproline. The value of 8.55% obtained was in good agreement with other values reported in the literature for fish gelatins (*2, 4*). Using this value the gelatin content of each extract from herring solubles was calculated from its hydroxyproline content.

Table I. Comparison of Solubles Prepared in Pilot Plant and Commercially by Using Acidification Process

	Pilot Plant Product, %	Commercial Product, %
Water	51.0	53.48
Total solids	49.0	46.52
Insoluble solids	4.93	7.18
Protein	39.16	35.37
Oil	0.95	2.20
Ash	9.87	8.94

Results and Discussion

To establish the degree of change in the properties of solubles prepared at different seasons of the year, solubles were produced in the pilot plant at approximately monthly intervals using the acidulation process. Viscosity determinations were carried out on each sample by both the Stormer and pour residue tests. The results of this study,

Table II. Comparison of Physical Properties and Analytical Data on Samples of Herring Solubles Prepared at Approximately Monthly Intervals

		Properties of Solubles				
				Pour Residue Test[b]		
		Stormer Values[a]		77° F.		50° F.
Month Caught	Insoluble solids, %	2 hr.	24 hr.	2 hr.	24 hr.	24 hr.
July	1.42	15.7	16.1	0.5	0.7	1.5
August	7.64	29.6	80.4	9.1	14.5	28.3
September	6.72	107.0	268.0	19.8	32.0	45.6
November	8.83	[c]	[c]	54.9	34.1	100.0
December	6.47	279.0	[c]	36.1	41.5	100.0
January	7.36	236.0	350.0	14.1	27.7	100.0

[a] Seconds/100 revolutions/200 grams at 77° F. Values determined at indicated times after preparation of solubles.

[b] Per cent of solubles retained in beaker at indicated temperatures and times after preparation of solubles.

[c] Too strong a gel for Stormer test to be applied.

recorded in Table II, show clearly the increased viscosity and gelling in solubles from winter-caught herring. Although the results suggest that some lowering in viscosity occurred after preparation of the November sample, it is unlikely that the differences shown are significant. Not only can viscosity values in these samples not be determined with great accuracy when the viscosities are so high, but also differences in the amount of heat applied could account for variations in the strength of the gel in these samples of solubles.

As insoluble solids are known to increase the viscosity of solubles (*13*), the rise in this component between July and August could perhaps have accounted for the viscosity differences noted in the samples from these two months. Because only minor changes in the insoluble solids levels occurred between August and January, however, this factor cannot explain the considerable differences in viscosity values obtained after August.

The extent of gel formation was somewhat greater in the samples prepared experimentally than was normally observed in those obtained commercially, probably because of a more efficient evaporator in the pilot plant system, resulting in the application of less heat to the stickwater than would normally be applied under industrial conditions.

The progressive increase in gel formation in solubles as the season progressed from summer to winter appeared to parallel the development of the gonads in herring. Stickwater was prepared from whole late season herring and from late season herring of the same batch from which the milt and roe had been removed. Results showed that the removal of milt and roe did not decrease the viscosity, but actually appreciably increased the Stormer values of the solubles.

In a further attempt to elucidate the factors involved in the viscosity changes of solubles, a pool of primary stickwater prepared from herring caught in February was divided into five parts and subjected to various treatments to evaluate their effect on the physical properties of the resulting solubles (Table III). As would be expected, the untreated stickwater produced solubles of high viscosity. The effect of removing insoluble solids completely by high speed centrifugation was determined. Untreated primary stickwater was centrifuged in a Servall SS-1 angle head centrifuge (Ivan Sorvall Inc., Norwalk, Conn.) at 20,000 × gravity for 20 minutes. The clear, supernatant liquid obtained was concentrated without further treatment. The product formed (sample 2, Table III) did not have properties appreciably different from those of the product from untreated stickwater. Lowering the pH of the stickwater to 5.0 followed by centrifugation resulted in a very significant decrease in viscosity as determined by the Stormer test. This latter treatment is essentially that used in the acidulation process (*10*). The product still gelled, however, at 50° F., as indicated by the 100% retention obtained in the pour residue test at that temperature. Perhaps not all the protein had coagulated at pH 5.0, but a further lowering of the pH to 3.9 followed by centrifugation did not produce any significant changes in either the Stormer or pour residue values of the solubles obtained. Digestion with a commercial proteolytic enzyme, however, reduced the viscosity to very low levels as determined by the Stormer test and completely eliminated gel formation—i.e., a protein was responsible for the viscosity effects. If the gelling agent was a protein it would have to be one which was soluble in water and dilute salt solution and was not coagulated by heat or acid. Gelatin has such properties, therefore it seemed not unlikely that this substance might be responsible for the gelling effect.

To investigate the possibility that seasonal variations in the gelatin content of solubles might be responsible for the viscosity differences observed, solubles were prepared from herring caught, as nearly as possible, at monthly intervals over a period of a year. Quantitative estimations of the gelatin content of the solubles were made and the results obtained were compared with the viscosity characteristics of the corresponding solubles (Table IV). The marked drop in the gelatin content of the solubles between March and April and a corresponding decrease in the viscosity of the solubles coincided with the spawning of the herring.

As the changes in the gelatin content of the solubles correlated so well with the seasonal changes in the viscosity of the solubles, gelatin is probably responsible for the variations in the product when the acidulation procedure is used in its preparation.

Tests were run to find which of a number of proteolytic enzymes, commercially available, would be most suitable for use in treating stickwater. Primary stickwater prepared from February herring was used as the substrate. In one case, this stickwater was acidified to pH 5.0 and the acid coagulable proteins were removed by centrifugation. The various proteolytic enzymes were then tested for their ability to destroy the gelatin in the supernatant solution. In another experiment the primary stickwater was used as the substrate without preliminary acidulation. This test was thus designed to demonstrate the capacity of the proteolytic enzymes, not only to attack gelatin, but also to break down proteins coagulable by acid. As the enzymes most active in attacking

Table III. Effect of Treating Stickwater in Various Ways on Physical Properties of the Solubles Produced

Sample No.	Treatment before Centrifugation	Stormer Values[a] 2 hr.	Stormer Values[a] 24 hr.	Pour Residue Test[a] 70° F. 2 hr.	Pour Residue Test[a] 70° F. 24 hr.	Pour Residue Test[a] 50° F. 24 hr.
1	Untreated	198.7	352.3	11	19	100
2	Centrifugation	136.3	551.1	4.8	9.3	100
3	pH to 5.0, centrifugation	30.2	53.2	0.8	1.3	100
4	pH to 3.9, centrifugation	36.5	48.5	0.2	0.3	75.7
5	Enzyme digested[b]	17.4	17.9	0.8	1.4	3.0

[a] See Table II.
[b] Rhozyme B-6, 0.5% level, pH 6.0, 140° F. Digestion period, 2 hours.

Table VI. Effect of Length of Time of Enzyme Treatment of Stickwater on Viscosity of Solubles

Time of Enzyme Digestion,[a] Minutes	Viscosity of Solubles: Stormer Values[b] 2 hr.	Stormer Values[b] 24 hr.	Pour Residue Test[c] 77° F.	Pour Residue Test[c] 50° F.
Control	199.0	241	22.5	100.00
30	33.1	43.0	12.5	19.90
60	26.8	29.7	5.9	20.70
120	21.4	25.6	6.2	15.90

[a] Rhozyme B-6, 0.5% level, pH 6.0, 140° F.
[b] See Table II.
[c] Determined at indicated temperatures 24 hours after preparation of solubles.

Table VII. Effect of Holding Stickwater at 212° F. for Varying Periods on Viscosity of Corresponding Herring Solubles

Sample No.	Length of Heat Treatment of Stickwater, Minutes	Stormer Values of Solubles[a]
1	0	62.1
2	<1	43.3
3	30	41.8
4	60	41.3
5	120	33.2

[a] Determined at 77° F. 2 hours after preparation of solubles.

the gelatin were also most active in lowering the viscosity when used on the unacidified stickwater, only the latter results, as they are of more general significance, are shown in Table V. Each enzyme selected was tested under its optimum conditions as recommended by the manufacturer. There was no correlation between the capacity of the enzymes to release α-amino groups and their ability to reduce the viscosity of the solubles.

For the most economical use of an enzyme preparation, it is desirable to know the minimum time required for a given concentration of enzyme to exert its maximum activity. The results in Table VI show that a 1-hour digestion period produced satisfactory solubles, from a viscosity standpoint, when a Rhozyme B-6 preparation was used at a 0.5% concentration.

As the presence of increased amounts of gelatin in the solubles, from late season herring, appears to be responsible for the high viscosity of solubles prepared by acid treatment, methods other than treatment of the stickwater with proteolytic enzymes might conceivably be effective in reducing the viscosity. The capacity of gelatin to produce a stable gel can be reduced by prior heat treatment of the gelatin solution (*9*). Consequently, the application of heat to stickwater before the preparation of solubles was investigated as a possible means of reducing the viscosity of the latter. Primary stickwater, pH 5.0, was heated to 212° F. for 30, 60, and 120 minutes (Table VII). The heated stickwater was cooled and then centrifuged, for 20 minutes, at 12,000 r.p.m. in the Servall SS-1 centrifuge. To avoid using different amounts of heat during the concentration of the heat treated samples of stickwater, the use of heat at this step was eliminated, by concentrating the samples at room temperature. A rotary evaporator with the receiver for the distillate chilled in an acetone–dry ice mixture was used.

The most pronounced reduction in viscosity occurred during the initial few seconds; however, heating at 212° F. for 2 hours failed to bring the viscosity of the solubles to the acceptable reading of 30 seconds by the Stormer viscometer test. A similar test at 185° F. instead of 212° F. presented the same picture, except that the total decrease in the viscosity of the solubles was less. Prolonged heating at the same temperature had little, if any, additional effect on the viscosity of the product.

Table IV. Relation between Gelatin Content and Viscosity of Herring Solubles Prepared from Herring Caught at Approximately Monthly Intervals

Month Caught	Gelatin in Solubles, %	Viscosity of Solubles: Stormer Values[a] 2 hr.	Stormer Values[a] 24 hr.	Pour Residue Test[a], 50° F., 24 hr.
August	1.18	20.8	21.8	6.74
September	1.62	28.0	42.1	100.00
November	2.26	63.0	94.0	100.00
December	2.31	128.0	136.0	100.00
January	2.68	>150.0	>150.0	100.00
February	2.56	138.0	>150.0	100.00
March	2.42	>150.0	>150.0	100.00
April	0.99	23.0	23.6	7.85
May	0.99	20.3	20.7	6.35
June	0.91	23.3	24.2	7.10

[a] See Table II.

Table V. Effect of Treating Primary Stickwater with Proteolytic Enzyme Preparations on Viscosity of Resulting Solubles

Enzyme Used[a]	Enzyme Concentration[b]	Pour Residue Test on Solubles[c]	α-Amino N Released in Stickwater[d] 0.5 hr.	1 hr.	2 hr.
Control	0	100.00	0	0	0
Rhozyme B-6	0.5	7.7	0.0470	0.0470	0.0504
	0.05	23.0			
	0.005	26.3			
Ficin	0.5	27.5	0.0924	0.1064	0.1288
	0.05	37.2			
	0.005	100.0			
Bromelin	0.5	12.0	0.1400	0.1428	0.1484
	0.05	37.4			
	0.005	25.4			
Protease	0.5	14.8	0.1456	0.1652	0.2184
	0.05	17.0			
	0.005	25.1			
Papain	0.5	26.0	0.0010	0.0212	0.0500
	0.05	39.7			
	0.005	100.0			
Collagenase	0.5	13.30	0.0200	0.0414	0.0506
	0.05	26.60			
	0.005	31.00			

[a] Stickwater treated with each enzyme for 2 hours under following conditions: rhozyme B-6, pH 6.0, 140° F.; ficin, pH 5.0, 104° F.; bromelin, pH 5.0; 140° F.; protease, pH 8.0, 104° F.; papain, pH 5.0, 98° F.; collagenase, pH 6.5, 98° F.

[b] Expressed as per cent of total solids present in stickwater.

[c] Determined at 50° F., 24 hours after preparation of solubles.

[d] Expressed as mg./ml. and determined at indicated times during digestion.

Conclusions

The source of the gelatin from herring and the reason that its increase in solubles should parallel the development of milt and roe in the fish have formed the subject of a separate investigation which will be reported elsewhere.

Proteolytic enzyme treatment has been shown, in this study, to be capable of eliminating the capacity of solubles to gel. As might be expected from the heterogeneous nature of a substrate which consists of all the suspended and dissolved proteins extracted from the fish during cooking, there appears to be little to choose between available enzymes in so far as their capacity to carry out the digestion is concerned. The lack of correlation between α-amino nitrogen release and viscosity change in solubles can be explained either by differences in ratio of endopeptidase to exopeptidase activity in the various enzyme preparations or in their capacity to attack non-gelatin proteins as compared to gelatin. It would seem that the relative cost of the various preparations would have a considerable bearing on the selection of an enzyme for this particular process. Because enzyme preparations can vary in activity from batch to batch, any doubts concerning the relative merits of different preparations should be resolved by direct comparison of activities on the substrate to be treated.

Heat treatment of acid treated stickwater has been found to lessen the effect of the gelatin, but not to the extent desired at the temperature tested. The results obtained in this study would tend to support a report (*13*) that the temperature rather than the duration of heating is the important factor in reducing the gelling capacity of gelatin. Although higher temperatures than those tested here might very well eliminate the effect of the gelatin, it would be necessary to determine whether or not such temperatures also affected the heat-

factors important to the nutritional value of the solubles.

As solubles are used as supplements in animal feeds, consideration should be given to the relative nutritional value of solubles prepared by acid and enzyme treatment of stickwater. When the two types of solubles were used to supplement basal natural and synthetic rations for the growth of chicks, no significant differences in the nutritive value of the products could be detected (*11*).

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THE GROWTH RATE OF *BANKIA SETACEA* TRYON

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Probably because of the difficulties involved in the determination of the rate of growth of marine wood borers, the literature on this subject is not extensive. Isham, Moore, and Smith (1951), who studied the growth of *Teredo pedicellata*, have adequately reviewed the literature to that date. Atwood and Johnson (1924) published a radiograph of shipworm-infested wood and thereby indicated the possibility of a technique for the study of the rate of growth of shipworms. Among those who have used this method are Ralph and Hurley (1952), who in New Zealand used X-rays in the determination of the rate of growth of *Bankia australis*. Crisp, Jones, and Watson (1953) demonstrated the use of X-ray stereoscopy for examining the infestation of shipworms *in vivo*, and Trussell, Greer, and LeBrasseur (1956) used radiographs to study short term (two months) growth of *Bankia setacea* in British Columbia. Taylor (1956) used the same technique to examine the effect of various preservatives in preventing shipworm and gribble attack on Douglas fir plywood.

Other studies on the growth of *Bankia setacea* were carried out by Kofoid and Miller (1927), who determined the rate in San Francisco Bay by measuring the burrows in test blocks exposed for known periods. The average rate ranged from 2.3 cm per month for specimens $1\frac{1}{2}$ months old to 6.3 cm per month for an individual 8 months old. Johnson and Miller (1935), in a fouling study at Friday Harbor, Washington, found an average rate of 1 cm increase in burrow length per month. To obtain more specific information on the growth rate of *Bankia setacea* in British Columbia waters, the present study was undertaken, and a preliminary report has already been given (Quayle, 1956).

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Methods

The investigation was carried out at the Provincial Shellfish Laboratory, Ladysmith, British Columbia. Eight fir blocks 3/4 x 5 5/8 x 12 inches were covered with fiber glass except for the ends, so that attack would occur only in that area. Four more untreated blocks were interspersed among the fiberglass blocks, and the whole group was suspended in a frame at a depth of 3 feet below the surface from the laboratory float. No controls were used.

Following the first immersion in November, 1954, the blocks were removed at monthly intervals and X-rayed at a distance of 30 inches, with 43 K.V., 4 milliampere seconds, 14 x 17 Kodak B.B. film, Par speed screens, half wave rectification, on a General Electric D-38 25 M.A., 85 K.V. self-rectified tube.

Length measurements were made directly on the burrows as shown on the radiographs. The greatest diameter, taken usually just behind the valves, the length of the pallet stalk, the total length of the pallet, and the number of cones were recorded (table 1). The volume of the burrow, admirable though it is as a measure of growth, was not used.

Results

The first X ray was taken on December 15, 1954, and showed that twenty-seven shipworms had entered the ends of six of the twelve blocks. The mean length at this time was 10.5 mm, with a range of 5 to 7 mm, indicating a slight variation in the time of settling. Further attack did not occur until seven more shipworms were apparent on the April radiographs. The data in table 1 were calculated from the measurements of the initial group of twenty-seven specimens, which by July had been reduced to five by mortality. The shipworms in the block with the heaviest infestation (initially eleven, and later thirteen) died between the April and May measurements as shown by the disposition of the valves and pallets. By the June measurement all in this block were dead, indicating a life span of only 6 to 8 months under fairly crowded conditions.

Length

As shown in figure 1, with water temperatures below 10° C the growth rate is relatively low, and about 4 months were required to attain a length of 200 mm; at temperatures above that

TABLE 1

GROWTH RATE OF *BANKIA SETACEA* IN LADYSMITH HARBOUR 1954-55

Date	Mean Monthly Temperature °F	Mean Body Length in mm	Range	Mean Body Diameter in mm	Range	Total Pallet Length in mm	Range	Pallet Stalk Section Length in mm	Pallet Blade Section Length in mm	Stalk Ratio Blade	Number of Cones	Range	Number of Animals
1954 December	45.0	10.5	5-17	2.2	1.5-2.5								27
1955 January	43.0	36.9	12-66	4.5	3.0-6.0								27
February	43.0	86.4	33-139	6.1	5.0-8.5	14.4	8-20	5.7	8.7	.660	5	4-8	27
March	44.0	139.6	61-207	7.6	5.5-9.0	19.0	12-25	7.6	11.5	.669	7	5-8	27
April	46.0	223.1	130-331	9.3	8.5-10.0	26.2	19-32	9.2	17.0	.543	9	7-11	27
May	54.0	340.3	255-465	10.1	9.0-12.0	34.4	32-40	12.8	23.0	.556	12	10-14	17
June	62.0	419.4	300-565	10.4	9.0-12.0	41.7	37-48	14.2	27.5	.517	15	14-17	13
July	66.0	505.0	430-570	12.0	12.0	50.0	48-59	16.0	34.0	.467	19	18-22	5

level a length of 300 mm was attained in 3 months. The greatest mean monthly increments (fig. 2) occurred in April, May, and June, with 120 mm in May. This may be owing to increased water temperature, to the relatively greater growth rate of larger animals, or to the increased availability of planktonic food. In connection with the latter possibility it should be noted that the spring phytoplankton bloom begins in Ladysmith Harbour normally in March, with maximum standing crop in April and May (Quayle, 1953).

The growth curves for three individual animals (1A, 7A, and 12B) that settled in the March-April period are shown in figure 3. Here, in spite of the smaller size, the initial growth rate is similar to that of the older groups, but it is greater thereafter. The second group showed a greater initial growth rate than the first, and this is no doubt due either to temperature or to availability of food. The mean length of 505 mm in July gave a mean monthly rate of 63 mm for this second group. The largest specimen (1A, fig. 3) was one of the second group and attained a length of 610 mm in about 5 months or an increment of 122 mm per month.

One of the blocks contained twelve shipworms, and, when the growth rate of those was compared to that of fifteen animals in five blocks, the former was found to have the highest rate. By April the single block group had a mean length of 50 mm more than the other group, although the initial size of the latter was slightly less than the group of twelve.

It should be mentioned that, since the burrowing for the most part was along the grain from the beginning, optimum conditions were favorable for maximum growth.

Diameter

In general, the rate of increase in the diameter of the burrow was similar to that of the length (fig. 1). When, however, the diameter is plotted against length rather than age, the relationship is somewhat different. There is at first a rapid increase in diameter up to a length of 50 mm, and from then until 250 mm the increase is only moderate. From a length of 250 mm to 400 mm the diameter increases more rapidly although the increase is not comparable to the initial rate. The greatest diameter attained was 12 mm.

Pallets

The data on pallet growth rate is not too satisfactory, for the

degree of breakage at the blade end is unknown. The pallet stalk, blade, and number of cones, following the terminology of Clench and Turner (1946), were recorded. The pallet as a whole has a growth rate pattern similar to that of the length and diameter of the burrow. The length of the blade and the number of cones increase markedly with age while the stalk portion increases very slowly, although this is the growing area of the pallet where new cones are added. The largest number of cones found was twenty-four, but it is known that breakage occurred in handling during the measuring, so that the counts in older animals are low. As would be expected, the rate of increase in the number of cones follows closely that of the length of the blade.

Summary

The X-ray technique appears to be a simple and satisfactory method for the determination of growth rates (burrow dimensions) of shipworms. X-ray stereoscopy described by Crisp, Jones, and Watson (1953) may have assisted somewhat, but in this case little difficulty was experienced in identifying the separate burrows.

Either burrow length or size of pallets appears to be a good measure of growth, since these do not vary with the spawning condition of the animal, and the variability due to differential shrinkage is not involved.

The results indicate a rate of growth considerably in excess of previous growth measurements for this and other species of Bankia. A mean monthly increase of 122 mm was attained by one specimen, which reached a length of 610 mm (24 inches) in about 5 months.

Length, greatest diameter, and pallet length growth rates show the same general trend.

The degree of crowding experienced in this study did not appear to affect the growth rate, although it did seem to affect the life span.

A much more complex experiment than the one reported here is required in order to demonstrate the possible variations in growth of *Bankia setacea* with a range of age, size, temperature, feeding, and degrees of crowding.

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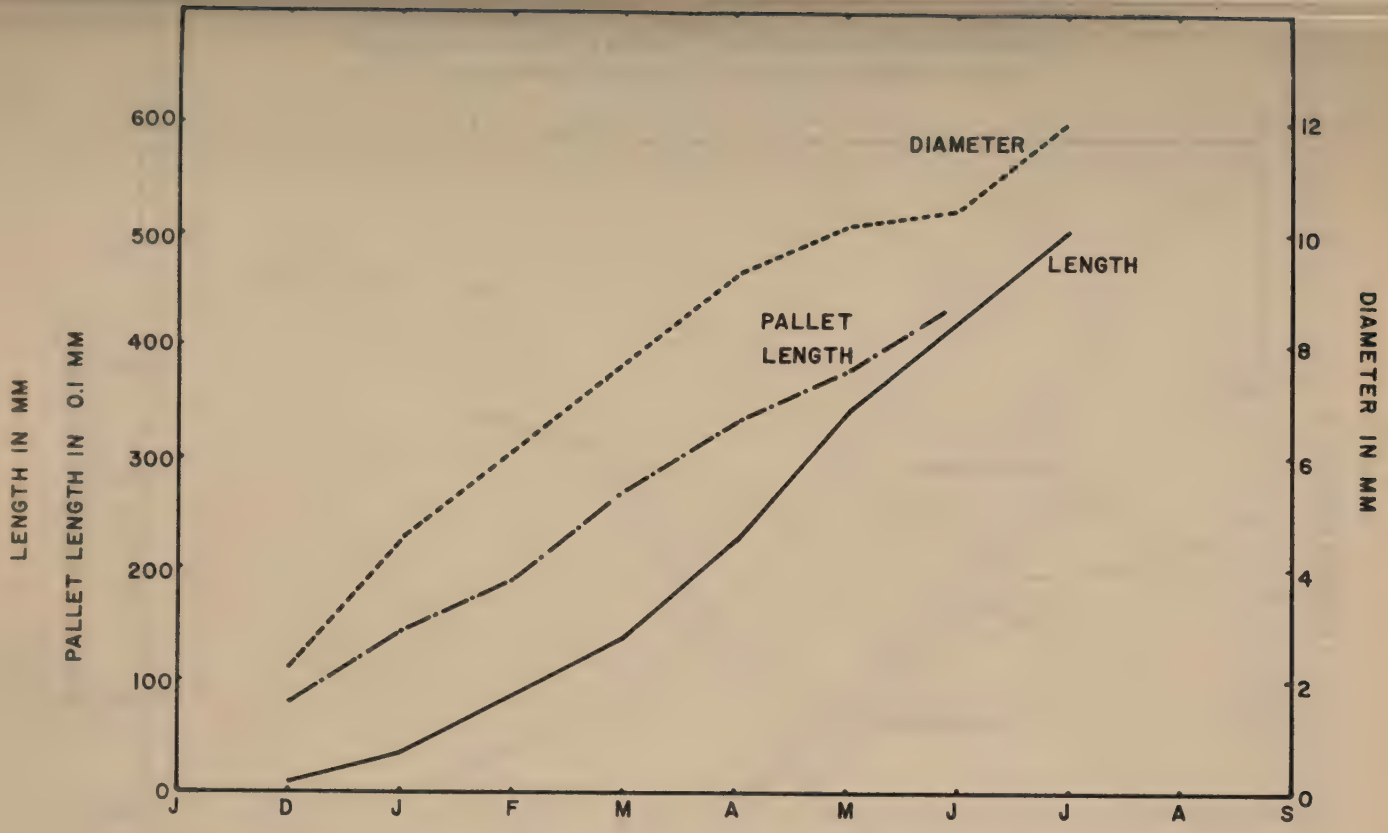

Fig. 1. Monthly growth in length, diameter, and pallet length of *Bankia setacea*, Ladysmith Harbour, 1954-55

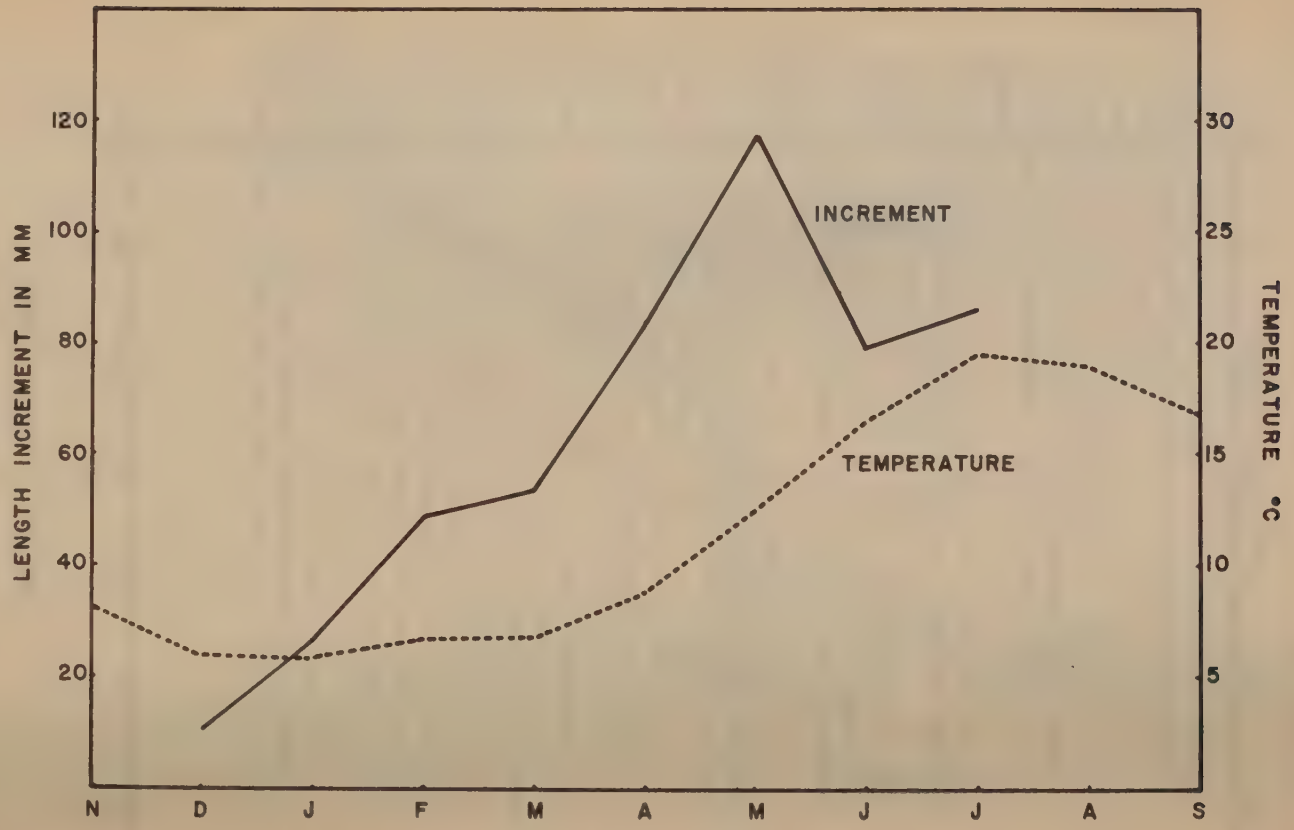

Fig. 2. Mean monthly length increment of *Bankia setacea*, Ladysmith Harbour, 1954-55. Temperature by thermograph, 0.91 meters deep

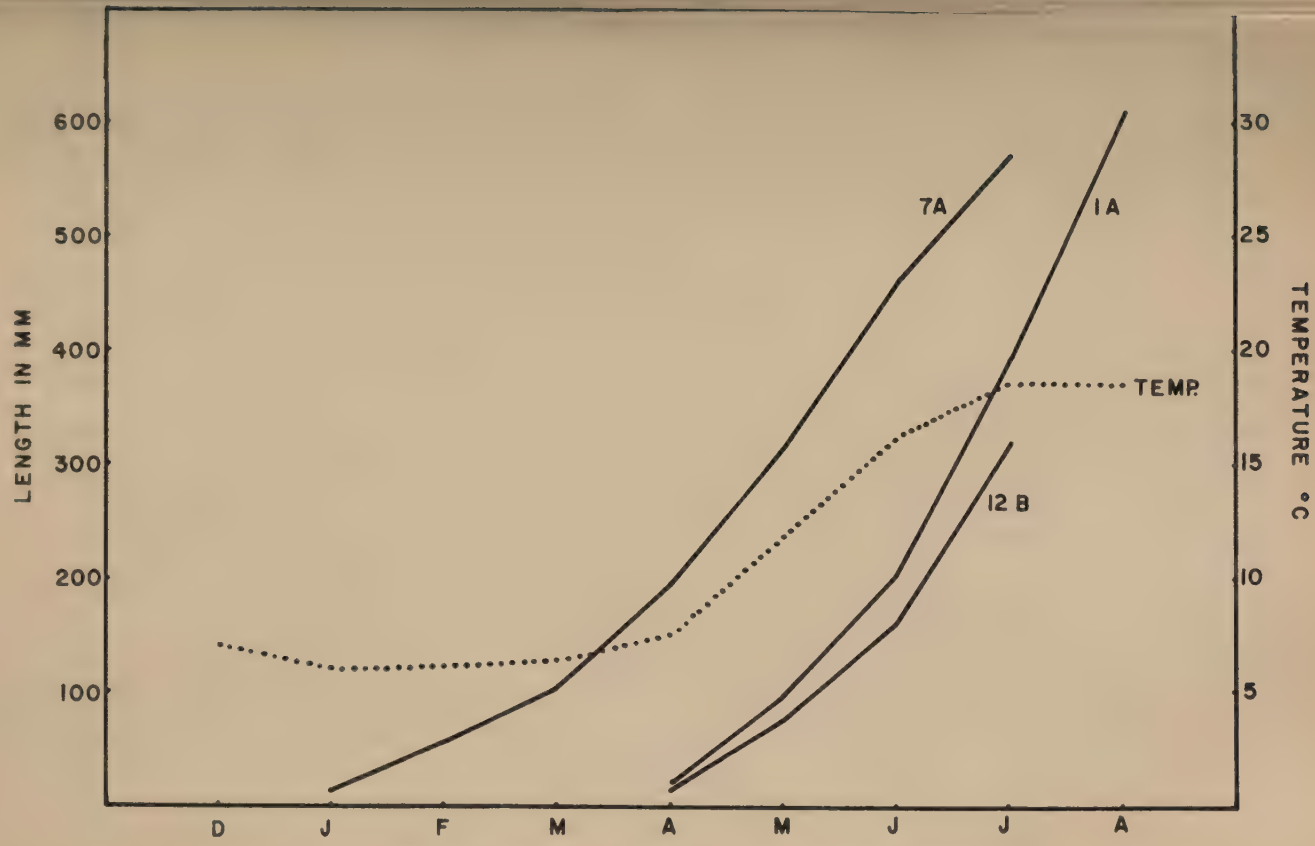

Fig. 3. Monthly growth in length of three selected specimens (1A, 7A, 12B) of *Bankia setacea*, Ladysmith Harbour, 1954-55. Temperature by thermograph, 0.91 meters deep

THE EARLY DEVELOPMENT OF *BANKIA SETACEA* TRYON

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Although the indigenous shipworm (*Bankia setacea* Tryon) of the north Pacific is of considerable economic importance because of its ability to destroy marine wooden structures and saw logs, no complete investigation of its life history has been made. Emphasis of the work done has been directed mainly to establishment of the breeding season by test block studies.

Fraser (1923, 1925, 1926) studied distribution and breeding with its possible relationship to temperature and salinity. The San Francisco Bay Marine Piling Committee (1927) devoted only a small portion of its efforts to *Bankia setacea.*

Boynton and Miller (1927) and Miller and Norris (1940) studied digestion and digestive enzymes in this species. White (1929, 1930) studied breeding, particularly at Departure Bay, British Columbia. Johnson and Miller (1935), during a general fouling survey, obtained information on breeding at Friday Harbor, Washington.

Coe (1941) studied sexual phases in *Bankia setacea.* Neave (1943), Black (1934), and Black and Elsey (1948) studied the breeding period in British Columbia by means of test blocks. Similar studies have been carried out by the W. F. Clapp Laboratories (Brown, 1955) in Washington waters. Miller and Dempster (1951) investigated the effects of low salinity and fresh water on *Bankia setacea,* and Miller (1951) gave an account of the distribution of Bankia and other shipworms in relation to ecological conditions. Trussell, Greer, and LeBrasseur (1956) studied the chemical control of *Bankia setacea.* The Larva has been described by Quayle (1953), who later (1956) gave a general account of the species.

The present observations are almost entirely the result of field studies, and were largely incidental to a program designed

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to investigate the larval life of the Pacific oyster (*Crassostrea gigas*) in British Columbia breeding areas.

Methods

Identification of the larva presented no problem, for it is the only species of Teredinidae that occurs in abundance in British Columbia waters. *Xylophaga washingtoni* is rather rare with limited distribution.

Quantitative plankton samples were taken by means of a 1 1/4-inch Jabsco pump powered by the main engine of the Shellfish Laboratory vessel *Saxidomus*. Samples to a depth of 30 meters were possible, but generally the maximum depth of sampling was limited by the vertical distribution of oyster larvae, or, in the case of the Ladysmith investigations, by the depth of the water.

The volume of the samples, normally 72 liters, was measured by a Leeds type water meter placed in the discharge line. The samples were concentrated in a plankton net of No. 20 or No. 25 bolting silk. At times a net of monel cloth (No. 20) was used. A plankton bucket permitted adequate washing of the net.

To count, the supernatant fluid in the samples was decanted and the remaining plankton was poured into a channeled plastic counting cell of 125 ml capacity, a size which permitted adequate rinsing of the sample jar. All of the larvae of concern (oyster and shipworm) in the sample were counted. Only immediately after a major oyster spawning was subsampling necessary, and this was never so with shipworm larvae, whose numbers did not approach those of the oyster larvae. These on occasion reached a density of 500 early stage larvae per liter.

Since during the summer in Pendrell Sound waters there is relatively little plankton other than lamellibranch veligers and protozoa (flagellates), counting presented no particular problem.

The Larva

Bankia setacea is an oviparous species and the larva is planktotrophic with a long pelagic life (Quayle, 1953). From the occurrence of size groups in successive plankton samples, the duration of the veliger period in Ladysmith Harbour appears to be about 3 weeks at a temperature of 12° to 15°C. Coe (1941) suggested a swimming period of 4 weeks in southern California waters.

The swimming and crawling activities of settling teredinid larvae have been described by Isham and Tierney (1953), and those of *Bankia setacea* are quite similar. The action of the foot and subsequent shell movements suggest the sequence of digging actions of mature sand-burrowing lamellibranchs (Quayle, 1949).

There is initial byssal attachment by *Bankia setacea,* although the byssus thread is very fine and weak compared to that of most lamellibranch species. A byssus was not observed in *Teredo pedicellata* by Isham and Tierney (1953) although Sigerfoos (1907) described one for *Xylotrya gouldi.*

The form of the valves immediately after settlement becomes asymmetrical; part of the posterior edge of the prodissoconch of only the right valve atrophies by what appears to be a process of erosion. The right valve at one period is little more than half the size of the left. When the newly settled animal is removed from its burrow and placed with the anterior gape down, the anteroposterior axis tilts markedly to the right, owing to the reduced length of the right valve. The cause of this atrophy is unknown, but it may have functional significance for it aids in forming the anterior gape, allowing development and operation of the foot. At this time (at the stage of a single row of teeth) there is no permanent posterior gape. Associated with settlement there must be quite drastic anatomical changes, particularly in musculature. Histological study of the process is required.

The first cutting teeth develop on the anterior edge of the shell, and on a specimen from a test block exposed for 48 hours a single row of 14 teeth had developed, the largest 14 μ in length. By the time a length of 20 mm is reached, 8 or 9 rows of teeth have been formed.

Observations indicate that *Bankia setacea* is able to bury itself completely about 24 hours after it has begun to burrow. The formation of the calcareous cone typical of the Teredinidea (Isham and Tierney, 1953) begins almost immediately. Within 4 days the 2 siphonal apertures separated by a calcareous bridge are well formed, and within 7 days the whole cone may be completed. Some cones may protrude slightly above the wood surface, but most are even with or below it. The diameters of the inhalant and exhalant apertures of the cone are approximately 80 μ and 50 μ, respectively. The bridge between the apertures disappears about one month after formation.

Pallets

Pallets are first formed when Bankia is about 0.5 to 0.6 mm in length. The initial section is spoon-shaped and not unlike the form of the pallet of *Teredo navalis*. The first pallet section measures about 90 μ wide and 200 μ in length.

Breeding

Most information on the breeding time in shipworms has been derived from the seasonal incidence of attack on test blocks. This method is of considerable practical importance and does provide a measure of breeding success, for there may be spawning without settlement. Breeding may also be studied by the occurrence of larvae in the plankton, and if sampling is adequate a measure of breeding intensity (but not necessarily success) may be obtained. Larval mortality may alter the relationship between breeding intensity and breeding success.

In 1951 in Ladysmith Harbour a general study was carried out on the seasonal abundance of planktonic organisms, among which were the larvae of *Bankia setacea.*

Samples of 283 liters each were taken at weekly intervals at a single station at depths of 0.91, 3.05 and 6.1 meters (bottom). The data on the mean monthly counts of shipworm larvae with mean monthly water temperatures at the 0.91 meter level are shown in figure 1.

In 1955, also in Ladysmith Harbour, plankton sampling specifically to determine the seasonal and vertical distribution of *Bankia setacea* larvae was carried out. Samples of 85 liters were taken at weekly intervals from a single station at depths of 0.91, 1.82, 3.64, 7.28, and 14.56 meters. These data are also graphed in figure 1.

A main spring spawning peak in the two series is demonstrated, although about a month apart, when the water temperature (3 feet below the surface) was 10°C or below. The salinity range is small in Ladysmith Harbour and varies between 24‰ and 28‰. The two succeeding minor spawning peaks are well separated in time, but the numbers are small for much significance to be attached to them. Fraser (1923, 1926) and White (1929, 1930) obtained data showing a similar main spring breeding. In contrast, Johnson and Miller (1935) concluded that *Bankia setacea* settles most intensively during October, November, and December with a cessation in January and February, beginning again in March

and April and continuing off and on sporadically through the summer. They suggested that 7°C and 12°C are the limits for effective breeding in Puget Sound.

In San Francisco Bay, Miller (1926) and Kofoid and Miller (1927) considered the breeding season of *Bankia setacea* to begin in February when water temperatures are at a minimum for the year and to cease generally at the beginning of summer, with peak breeding in April or May. Coe (1941) stated that spawning occurs in this species in autumn and spring with resting periods in winter and summer in southern California.

Neave (1943), on the basis of test block studies, found maximum settling to occur during the months of September, October, and November in three different areas (including Ladysmith Harbour). He found no close relationship between the intensity of settlement and temperature or salinity. Black and Elsey (1948), also using test blocks in a number of areas along the British Columbia coast, found greatest infestation during the fall season, although at Shannon Bay, the area of heaviest attack, there was also a minor spring peak. Test blocks at Port Townsend, exposed during the period 1948-54, indicated breeding and settlement of *Bankia setacea* throughout the year according to Brown (1955). Trussel, Greer, and LeBrasseur (1956) found a fall and winter peak of settlement in Vancouver Harbour. The Pendrell Sound studies described in this account showed summer breeding.

These test block studies, which are widely spaced both in time and in place, show highly variable results and demonstrate the need for continuous long-term observations using both plankton and test blocks.

Vertical Distribution of Larvae

Ladysmith Harbour

In both the 1951 and 1955 investigations in Ladysmith Harbour, samples were taken at various depths to determine the vertical distribution of the larvae. These were day samples; consequently any diurnal or tidal effect would not be apparent.

In the 1951 series (193 samples), 4 per cent of the larvae were found at the 0.91 meter level, 32 per cent at the 3.05 meter level, and 64.4 per cent at the 6.1 meter depth. In 1955 (245 samples), 8 per cent of the larvae were found at the surface, 13 per cent at the 0.91 meter level, 16 per cent at the 1.82 meter

level, 35 per cent at the 7.28 meter level, and 28 per cent at the 14.56 meter level, which is near the bottom.

Pendrell Sound

The Pendrell Sound oyster studies presented a further opportunity to observe the vertical distribution of *Bankia setacea* larvae. The intensity and time of sampling varied from year to year according to the abundance of oyster larvae, but the technique was uniform and all samples were taken at Station 2 (Quayle 1957).

The condensed data for the years 1952-56 are shown in table 1 together with typical temperature and salinity distributions taken on the days designated in the table.

In years when considerable numbers of Pacific oyster larvae were present, series of samples were taken at various depths at Station 2 (total depth 20 meters) to determine the possibility of a diurnal movement. Samples were usually taken every 3 hours, at times of slack water and halfway between, for periods of about 3 days. Both oyster and shipworm larvae were counted. The results of two such series are shown in tables 2 and 3, and those for a 3-day period in 1956 are shown in figure 2 together with the tidal fluctuations.

The 1956 series of samples (tables 1 and 3) indicated a tendency for *Bankia setacea* larvae to approach the surface during the hours of darkness and to be found at greater depths during the day. The fact that this tendency was not well marked in the 1952 series (table 2), apparently because of the selection of sampling depths, led to the statement that no diurnal movements had been observed (Quayle, 1953).

The data in table 2 show the larvae to be concentrated at the 7-meter depth and below in the day samples, while in the combined day and night samples, particularly those of 1956 (table 3), the upper level of larval concentration lies between 3 and 4 meters. In 1956 the night samples contained 4.5 times more larvae than did the day samples. No light intensity measurements were made, but Secchi disc readings during the summer in Pendrell Sound vary between 8 and 12 meters. A tendency for the larvae to be concentrated below the halocline is also indicated. Salinity stratification occurs in Pendrell Sound during late spring and summer as a result of river runoff from large rivers in Toba Inlet (Quayle, 1957). There is no evidence of a temperature barrier. That larvae are found at depths below those sampled in the described series is shown by their presence in samples taken

TABLE 1

BANKIA SETACEA: VERTICAL DISTRIBUTION OF LARVAE WITH TYPICAL TEMPERATURE AND SALINITY VALUES AT THE SAMPLING DEPTHS

Depth (meters)	‰ Larvae	Temp. °C	‰ Salinity
	1952. Aug. 15		
0	...	22.5	17.3
.91	1	22.4	17.3
3.05	7	19.7	24.7
6.1	35	18.6	25.7
12.2	57	14.7	27.2
	124 samples Aug. 12-Aug. 26 (day and night)		
	1953. Aug. 9		
0	0	21.7	18.8
.91	0	21.6	18.8
3.66	3	18.1	25.3
7.32	50	13.7	27.2
10.98	47	12.2	27.8
	60 samples Aug. 2-Sept. 8 (day)		
	1954. Aug. 2		
0	0	21.6	17.1
.91	0	21.3	17.8
1.82	0	20.7	17.8
3.64	14	17.0	25.1
7.28	61	14.7	26.8
28.92	35	13.2	27.6
	54 samples July 24-Aug. 30 (day)		

from 25 meters with a Clarke-Bumpus sampler, and in other pumped samples from the same depth.

As shown in figure 2, there appears to be some correlation between tidal level and the occurrence of *Bankia setaceà* larvae. In the July 26-28 series of samples, however, the tidal pattern was quite different from that during July 16-19, and there is no indication of a similar correlation in the later series.

TABLE 1 (cont.)

Depth (meters)	‰ Larvae	Temp. °C	‰ Salinity
	1955. Aug. 4		
0	0	19.0	19.2
.91	0	19.0	20.0
1.82	0	19.0	19.3
3.64	0	18.8	20.6
7.28	10	17.8	24.4
10.92	7	.....	.....
14.56	36	15.5	28.2
18.20	47	.....	.....
	53 samples July 27-Aug. 19 (day)		
	1956. July 17		
0	0	24.2	20.3
.91	0	23.0	20.1
1.82	1	22.0	20.5
2.73	7	20.5	21.7
3.64	35	18.5	24.4
5.46	27	16.5	25.8
7.28	30	15.2	.....
	137 samples July 16-19 (day and night)		
	1956. July 26		
0	0	21.8	18.6
.91	0	21.6	18.6
1.82	0	21.6	18.6
2.73	1	20.4	23.8
3.64	8	17.1	25.6
4.55	28	14.9	26.5
5.46	63	14.2	26.7
	161 samples July 25-28 (day and night)		

Also test blocks exposed at Station 2 in 1954 caught one *Bankia setacea* per 6.5 sq cm (1 square inch) at the 3.6 meter level, 10 per 6.5 sq cm at 7.3 meters, 25 per 6.5 sq cm at 12.7 meters, and 42 per 6.5 sq cm at 16.4 meters. On the other hand, in

TABLE 2
NUMBERS OF *BANKIA SETACEA* LARVAE IN SAMPLES OF 42 LITERS (3 CUBIC FEET), STATION 2, PENDRELL SOUND, AUGUST, 1952

Date	August 12		August 13			
Time	17:10	23:20	06:48	14:30	18:47	24:00
Depth in feet 3	2	3				
10	3	644				13
20	570	19	5	81	119	131
40	152	6	190	93	56	16
Total	727	672	195	174	175	160

Date	August 14				August 15		
Time	07:32	12:40	15:40	20:43	08:51	17:12	22:00
Depth in feet 3							
10		9	1			5	40
20	7	231	14	60	14	12	47
40	133	7	40	33	168	219	23
Total	140	247	55	93	182	236	110

Pendrell Sound oyster larvae are concentrated above a depth of 3 meters.

Discussion

Of interest is the apparent discrepancy between the planktonic larval and the test block studies in the breeding time of *Bankia setacea*. It is unfortunate that no test blocks were exposed during

TABLE 2 (cont.)

Date	August 16			August 23
Time	01:30	09:40	17:47	20:20
Depth in feet 3				0
10	32		3	5
20	86	19	26	15
40	13	64	115	16
Total	131	83	144	36

Date	August 24				August 25			
Time	02:30	08:30	14:20	20:40	03:00	09:30	15:00	21:00
Depth in feet 3	19	2		1	4			1
10	12	1	1	7	6	1	1	2
20	0	4	2	10	2	2	1	7
40	1	7	7	5	9	7	2	12
Total	32	14	10	23	21	10	4	22

the period of the larval studies in Ladysmith Harbour, for the relationship between numbers of larval shipworms and intensity of settling requires investigation similar to that which has been done with oysters. For instance, it has been found that in Pendrell Sound approximately one advanced stage Pacific oyster larva per 3 liters will provide a set of at least one spat per 6.5 sq cm (1 square inch) of collecting surface.

In connection with the cause of the difference in time of settling demonstrated by the two methods, the number of observations is insufficient to determine the degree of significance. With the test block method itself, which has been applied fairly

TABLE 2 (cont.)

Date	August 26			
Time	04:00	10:30	15:30	21:30
Depth in feet 3	1			4
10	2	4		4
20	6	1		3
40	3	0	6	5
Total	12	5	6	16

extensively, the annual variation both within and between areas is considerable. The only test block study in Ladysmith Harbour (Neave, 1943) showed fairly intensive settling in the period from June to October in 1941 with the greatest attack in June. In 1942 settling occurred mainly in the September-November period with the maximum in October. In addition, results from a single station may be misleading, and more stations might be used to advantage, together with longer periods of observation.

In general, however, the data indicate that *Bankia setacea* breeds most frequently during periods of low temperature, although the larvae may occur in strata with wide temperature differences. That there are exceptions to this is shown in the Pendrell Sound data.

Other species of Bankia whose breeding periods have been studied are generally high temperature spawners. Ralph and Hurley (1952) demonstrated that *Bankia australis* in New Zealand in 1951 had a breeding peak at a temperature of about 20°C. Sigerfoos (1907) concluded that *Bankia gouldi* spawned during periods of high water temperature in the Beaufort area. A similar conclusion was reached by Scheltema and Truitt (1954), who showed that the same species settles most intensively in Chesapeake Bay in summer when the water temperature is well above 20°C, although these authors believe that actual spawning may begin at lower temperatures.

The apparent diurnal movement demonstrated for *Bankia setacea* may be due to light. Isham, Smith, and Springer (1951)

TABLE 3

NUMBERS OF *BANKIA SETACEA* LARVAE IN SAMPLES OF 72 LITERS (3 CUBIC FEET), STATION 2, PENDRELL SOUND, JULY, 1956

Date	July 16	July 17							July 18								July 19					
Time	23:00	01:00	05:00	08:30	13:00	18:45	21:00	23:20	01:40	15:40	09:40	13:30	17:30	19:45	22:03	24:00	02:30	06:10	10:00	14:00	18:00	Total
Depth in feet 0	1	1																				2
3																						0
6	1																3					4
9								8	1								18	9				36
12	9	74	10			5	6	29	155	23						99	21	0				421
18	n. s.	n. s.	n. s.		3	4	12	21	46	81	1	4	15	93	75	27	108	30	0	3	0	523
24	29	40	41	61	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	171
Total	40	115	51	61	3	9	18	58	211	104	2	4	15	93	75	126	150	39	0	3	0	

TABLE 3 (cont.)

Date	July 25		July 26							July 27							July 28						
Time	21:00	24:00	03:00	05:00	08:00	11:00	14:00	17:30	24:00	03:30	06:00	08:45	11:40	14:43	18:19	21:41	01:00	04:07	07:00	09:46	12:46	15:22	Total
Depth in feet 0																							0
3																							0
6																							0
9				2															3	1	1		7
12		31	1	0				1		2							3	6	0	2	0		46
15		42	9	7	3	1		10	15	1	12		1			20	26	38	0	0	4	2	151
18	4	54	59	22	0	0	1	27	13	24	21	2	9	0	6	36	20	62	12	6	21	14	417
Total	4	127	69	31	3	1	1	38	28	27	33	2	10	0	6	56	49	106	15	9	26	16	

have studied experimentally the effect of light on the movement of teredinid larvae (mainly *Teredo pedicellata*) and have concluded that under natural conditions the larvae will concentrate at the surface during darkness and during the day will be dispersed and tend to remain in the deeper, less illuminated water. Owen (1953) indicated that *Teredo norvegica* larvae tend to settle in increased numbers in dimly lit regions. Schwarz (1932) inferred that *Teredo navalis* exercises selection of the light-exposed north side of piling at Wangerooge. Vertical movements of other bivalve larvae have been indicated by Carriker (1951), Quayle (1952), and Yasuda (1952). Swimming rates of about 7.5 mm per second for *Teredo pedicellata* larvae found by Isham and Tierney (1953), if applied to *Bankia setacea,* would make it possible for this species to undergo the vertical movements indicated in the Pendrell Sound data.

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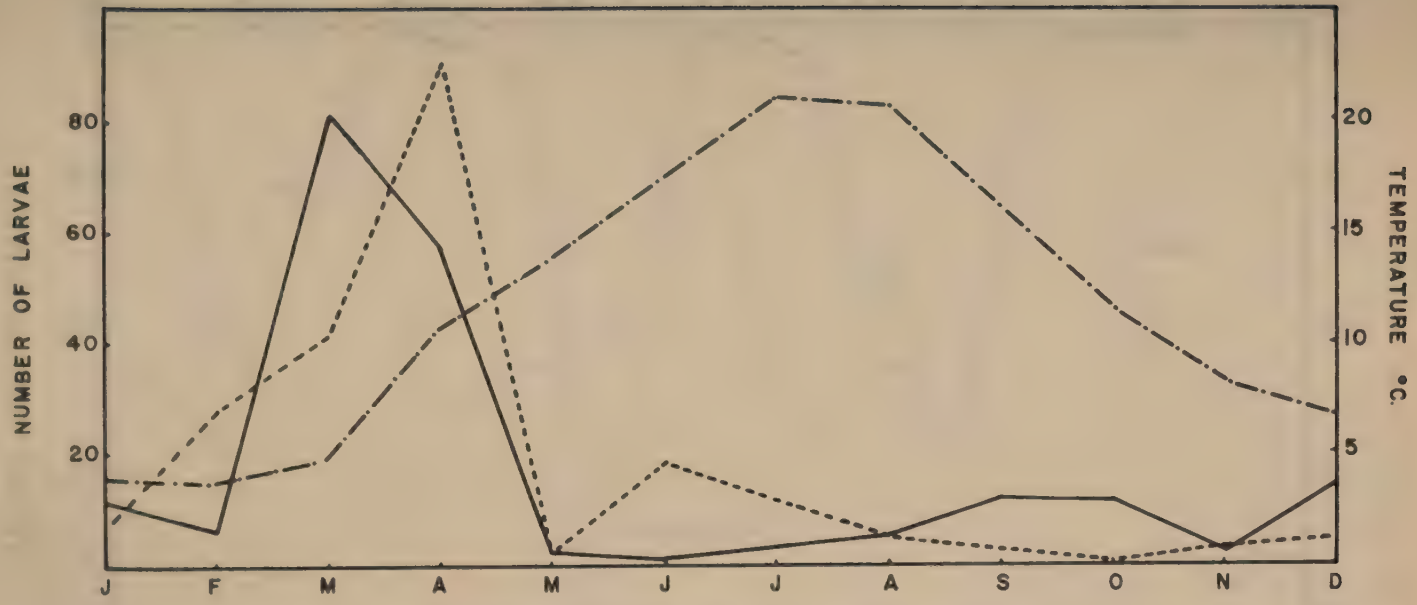

Fig. 1. Seasonal abundance of the larva of *Bankia setacea* in Ladysmith Harbour. (———) 1951; (-----) 1955; (–·–·–·–) thermograph temperature, 0.91 meters below surface

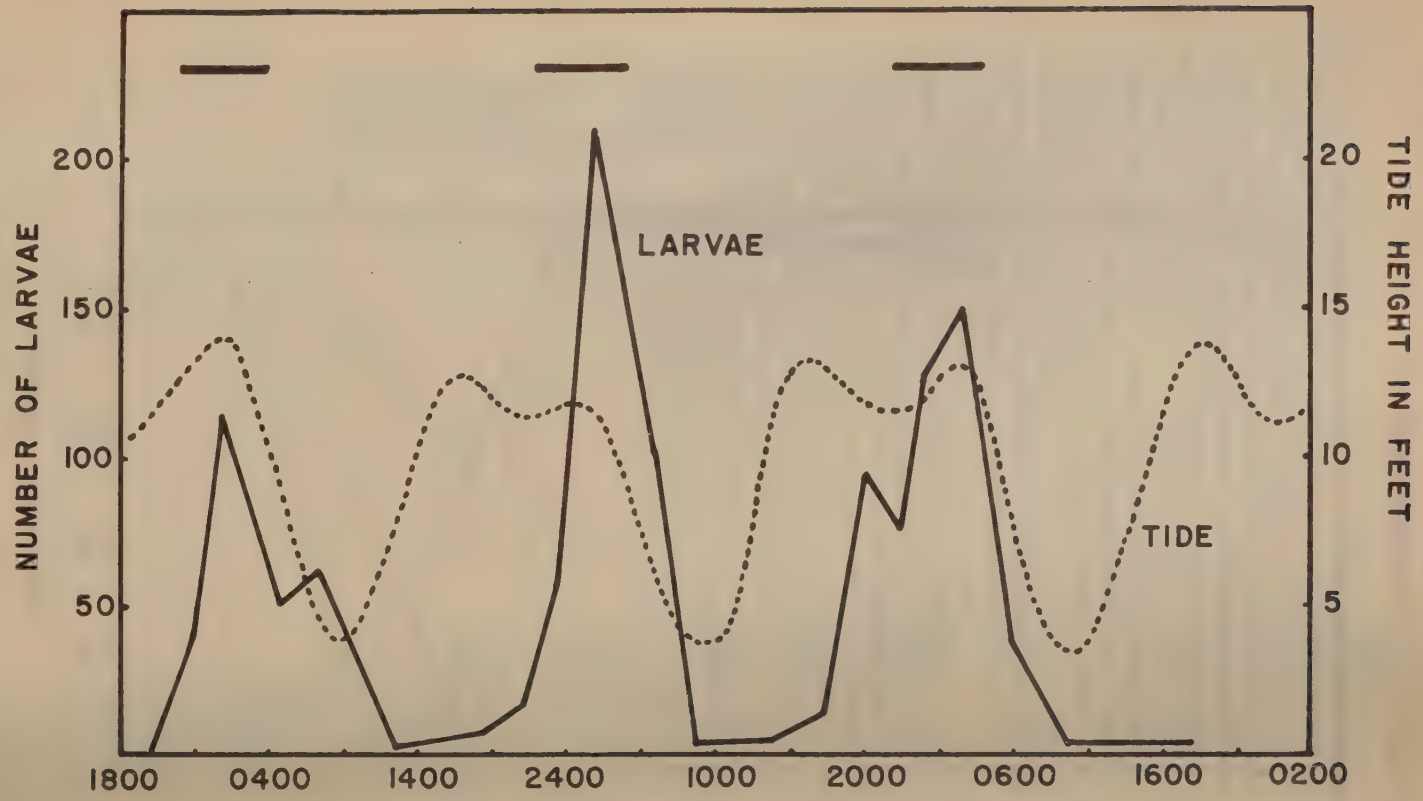

Fig. 2. Occurrence of *Bankia setacea* larvae (total in samples from 6 or 7 depths) of Station 2, Pendrell Sound, July, 16-19, 1956. Bars mark periods of darkness

ISOLATION OF PREGN-4-ENE-17α,20β-DIOL-3-ONE FROM THE PLASMA OF PACIFIC SALMON (ONCORHYNCHUS NERKA)

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Introduction

The isolation of cortisol, cortisone and 17-hydroxyprogesterone from salmon plasma and the high blood levels of the first two, particularly in post-spawned fish, was recently described (Idler et al., 1959). Three ultraviolet absorbing substances less polar than corticosterone were found. Fraction 6B, the substance of intermediate polarity when chromatographed in heptane:benzene 1:1-formamide (HBF), was 17-hydroxyprogesterone. The most polar of the three steroids, fraction 6A, has now been identified as pregn-4-ene-17α,20β-diol-3-one. To our knowledge this represents the first reported isolation of this steroid from a natural source.

Materials and Methods

Approximately 80 post-spawned female sockeye salmon, taken at Chilko Lake, B.C. in September, 1959, were bled by severing the caudal artery. Precautions were taken to exclude slime and excreta from the blood. Plasma (1640 ml) was prepared from the heparinized blood and frozen on dry ice. The thawed plasma was extracted 3 times with 2.5 volumes of CH_2Cl_2. The chilled extract was washed with 0.05N NaOH, 0.1N acetic acid and H_2O and the CH_2Cl_2 removed at 30° in vacuo. The residue was taken up in 70% methanol and non-steroid fatty material removed by partition with hexane.

Chromatography paper was pre-washed (Idler et al., 1959). Solvent

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systems used were hexane—propylene glycol (HPG) (Savard, 1953), hexane-ethylene glycol (HEG), benzene:hexane:methanol:water 33:66:80:20 (BHMW) (Bush and Sandberg, 1953) and HBF.

The C-20 epimers of pregn-4-ene-17α,20-diol-3-one were located on paper chromatograms with the aid of their absorption of ultraviolet light and their fluorescence under ultraviolet light after the paper was sprayed with 15% aqueous H_3PO_4 and heated at 90°C for 10 minutes. They were prepared by selective reduction with $NaBH_4$ of the C-20 ketone of chromatographically pure 17α-hydroxyprogesterone under conditions similar to those described for other corticosteroids (Norymberski and Woods, 1955). The 20α-epimer was also prepared by microbiological reduction of 17α-hydroxyprogesterone with Rhodotorula longissima. The conditions were as those described by Carvajal et al., (1959) except that 2-day old cells were used and 50 mg of steroid were added per 100 ml of Edamin-dextrose-cornsteep liquor medium and incubated at 25-26° on a rotary shaker.

Infrared spectra were recorded of micro KBr pellets, containing 20-50 μg of steroid, using a Beckman IR-4 equipped with a beam-condensing system.

Results

The new steroid had its max. at 240 mμ in methanol. It gave a negative test for the dihydroxyacetone side-chain (Silber and Porter, 1954) and could be detected on paper chromatograms with a modified Zimmerman reagent (Bongiovanni et al., 1957). The unknown and synthetic pregn-4-ene-17α,20β-diol-3-one both had an R_f relative to 17α-hydroxyprogesterone (R_{HP}) of 0.21 in HPG and 0.40 in HEG. The R_f of both substances was 0.18 in the HBF system.

The bismuthate oxidation products (Bush and Sandberg, 1953) of the unknown and the synthetic 20β-epimer both had an R_f of 0.58 in BHMW identical to that of androst-4-ene-3,17-dione and all three compounds gave positive Zimmerman reactions.

The unknown and synthetic pregn-4-ene-17α,20β-diol-3-one gave identical sulfuric acid chromogens with maxima and minima at 298 mμ and 238 mμ after

4 hours and 305 mμ and 242 mμ after 24 hours at room temperature.

The infrared spectrum of the unknown exhibited absorption maxima at 1612, 1660, 1333, 1263, 1231, 1183, 958, 943, 869 and 776 cm^{-1} characteristic of a Δ^4-3 ketone (Jones and Herling, 1954). The absorption intensity at 3460 cm^{-1} indicated more than one OH group in the molecule. The spectrum corresponded in all respects with the published spectrum of pregn-4-ene-17α,20β-diol-3-one (Dobriner et al., 1953) which was probably synthesized by the reduction of 17α-hydroxyprogesterone 3-benzylthioenol ether with $LiAlH_4$ (Romo et al., 1951). The infrared spectra of both products were identical with that of the synthetic 20β-epimer prepared by borohydride reduction of 17α-hydroxyprogesterone. The 20α-epimer preparations made by reduction of 17α-hydroxyprogesterone with $NaBH_4$ and with R. longissima gave identical infrared spectra which were quite distinct from the spectrum of the 20β-epimer.

From the free steroid extract of 1640 ml of post-spawned female plasma 14.4 μg/100 ml of 17-hydroxyprogesterone and 5.5 μg/100 ml of the 20β-dihydro-epimer were recovered following chromatography in the HPG system. The concentration of 17-hydroxyprogesterone was comparable to that found previously (Idler et al., 1959) in plasma of pre-spawned females. Plasma levels of these steroids in the two sexes at various stages of the spawning migration will be the subject of a future report.

Discussion

The reduction of 17α-hydroxyprogesterone with $NaBH_4$ gave two ultraviolet absorbing compounds which were readily separable in the HEG solvent system. The 20β-dihydro-epimer predominated (90-95%) as expected from previous findings with other corticosteroids, (Norymberski and Woods, 1955; Southcott et al., 1956). There was a possibility that the minor component arose from a contaminant in the 17α-hydroxyprogesterone and that the 20α and 20β-dihydro-epimers of 17α-hydroxyprogesterone were not separable in the HEG solvent system. This possibility was eliminated by the synthesis of the 20α-epimer using a stereospecific microbiological reduction of 17α-hydroxyprogesterone. After a

one-day incubation 80% of the added Δ^4-3-ketone was recovered by CH_2Cl_2 extraction of the media and cells without prior grinding. The ultraviolet absorbing products were pregn-4-ene-17α,20α-diol-3-one (95%) and unchanged 17α-hydroxyprogesterone (5%). The yield was appreciably greater than that reported for longer time reduction of steroids with a dihydroxyacetone sidechain under similar conditions.

The reduction of the C-20 keto group, without the reduction of the Δ^4-3-ketone, is well established in a variety of tissues and species but not in fish (Vermeulen and Caspi, 1959; Berliner et al., 1958; Wiest, 1959). Progesterone has been converted to pregn-4-ene-17α,20β-diol-3-one by testicular tissue (Lynn et al., 1958).

Since 17-hydroxyprogesterone occurs at a relatively high level in the plasma of salmon, and has not yet been found in the plasma of other species, it is the most logical precursor of the 20β-dihydro-epimer. The site of the transformation is not necessarily the liver or other tissues referred to above. Recently human adrenal incubates have been shown to produce 20α and 20β-dihydrocortisol (Touchstone et al., 1959). Adrenal vein blood of young calves contains pregn-4-ene-20α-ol-3-one and the concentration is much higher than in peripheral blood, indicating that it is an adrenal secretion (Balfour et al., 1959). The biological activity of pregn-4-ene-17α,20β-diol-3-one is not known to the authors. However, 20β-dihydrocortisone has the same activity as cortisone in the eosinophil test (Carvajal et al., 1959), 20β-dihydrocortisol is active in the glycogen assay (Abelson et al., 1955), and 20β-dihydroprogesterone has progestational activity (Zander et al., 1958).

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BIOCHEMICAL STUDIES ON SOCKEYE SALMON DURING SPAWNING MIGRATION

XII. LIVER GLYCOGEN[1]

VIOLET M. CHANG AND D. R. IDLER

Abstract

Liver glycogen levels were determined for a pure stock of sockeye salmon (*Oncorhynchus nerka*) taken at three locations during spawning migration. The liver glycogen content of the male was found to be consistently greater than that of the female throughout the entire river migration. In both sexes liver glycogen decreased during the earlier phase of migration, but increased during the later stage so that the levels at the spawning grounds were approximately twice those at the mouth of the river. The changes which occur are discussed in relation to sex differences, energy expenditures, and plasma steroid hormone levels.

Introduction

During the 1957 Fraser River sockeye salmon run to Stuart Lake, an extensive program was initiated at this Station to investigate the biochemical changes which occur during spawning migration (1). As part of this program the liver glycogen content at various stages of the river migration of the 1959 Chilko Lake migrants was studied and the results are presented in this paper.

Materials and Methods

The liver samples were obtained from the 1959 Chilko Lake sockeye salmon migrants at Albion (situated near the mouth of the Fraser River), at Siwash Bridge (358 miles upstream from Albion), and at the spawning grounds on the Chilcotin River. The fish at Albion were caught in a gill net and transferred to a "live" box which was anchored beside the boat. The fish were processed as quickly as possible. A dip net was used to capture fish at Siwash Bridge and a reef net on the spawning grounds. After the fish had been bled by severing the caudal artery, the livers were removed, immediately frozen in liquid nitrogen, and stored in separate polyethylene bags on dry ice. The frozen liver samples were individually powdered in a mortar with liquid nitrogen. Aliquots of approximately 10 g were placed in 100-ml beakers covered with aluminum foil and stored on dry ice until analyzed.

The livers were homogenized with trichloracetic acid (TCA) in a Servall omnimixer at a speed of 14,000 r.p.m. The first extraction was carried out with 30 ml of 10% TCA for 5 minutes, after which the mixture was centrifuged at 12,000 r.p.m. for 15 minutes. The supernatant fluid was decanted and the residue was extracted twice more with 30 ml of 5% TCA for 2 minutes. The supernatants from all extracts were combined and diluted to 100 ml. The procedure was carried out at 0° C.

The glycogen was precipitated by mixing 1 volume of TCA extract with 5 volumes of absolute ethanol in a stoppered conical centrifuge tube and

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refrigerating overnight. The precipitated glycogen was collected by centrifugation at 3000 r.p.m. for 15 minutes. The supernatant was discarded and the tubes were allowed to drain in an inverted position for at least 10 minutes. The residue was dissolved in a measured quantity of distilled water.

Glycogen was assayed with the anthrone reagent. The color was developed according to Carroll *et al.* (2) and read in an Evelyn colorimeter using a 620 mμ filter.

Results

The results of liver glycogen determinations on both male and female fish at various stages of spawning migration are shown in Table I.

TABLE I

Liver glycogen (mg/g liver (wet weight)) in migrating sockeye salmon at three locations

Albion		Siwash Bridge		Chilcotin River		
				Postspawned		Prespawned†
M	F	M	F	M	F	M
22.2*(1a)	2.57(1a)	1.15(2a)	0.83(2a)	21.9	2.31	45.5
19.1*(1a)	0.87(1a)	9.89(2a)	1.20	35.5	3.61	68.7
16.1	0.36*	7.93(2a)	1.58(2a)	41.8	4.40	33.4
11.1*	1.97*	4.76(2a)	2.52(2a)	63.1	3.90	54.1
15.1	0.40*	7.74	3.68	11.5	8.34	26.7
24.3*(1b)	4.93*(1b)	6.90	2.23(2b)	39.8	6.28	55.1
21.6*(1b)	3.45(1b)	7.98	1.20	8.6	0.21	63.7
16.8*(1b)	1.61*(1b)	6.63(2b)	1.54(2b)	39.6	7.12	18.0
12.0*	3.69*(1b)	5.84(2b)	1.88(2b)	35.4	7.56	36.9
9.7*	—	9.04(2b)	—	23.0	—	50.3
27.4	—	—	—	—	—	—
17.8±1.7‡	2.20±0.53	6.79±0.78	1.85±0.29	32.0±5.1	4.37±0.93	45.2±1.62

*Scale readings showed these samples were from Chilko Lake fish. All fish taken at Siwash Bridge and the Chilcotin River were Chilko Lake fish.

†These are presented as minimal values.

1. Samples obtained Aug. 6, 1959. (*a*) Analyses completed Aug. 12, 1959. (*b*) Analyses completed Aug. 19, 1959.
2. Samples obtained Aug. 29, 1959. (*a*) Analyses completed Sept. 10, 1959. (*b*) Analyses completed Oct. 26, 1959. These data indicate that no detectable loss of liver glycogen occurred in samples which had been stored up to 8 weeks. In almost all cases analyses were completed on samples within 4 weeks after they had been obtained.

‡Mean ± standard error.

NOTE: M = male; F = female.

It is probable that the variation of liver glycogen content in individuals was attributable in part to the degree of muscular exhaustion due to struggling of the fish during capture. However, Miller *et al.* (3) have determined in experiments with hatchery-reared rainbow trout that the liver glycogen of well-nourished fish was relatively stable to change during exercise even though muscle glycogen was depleted. The level of liver glycogen in the individual fish may therefore be regarded as an indication of its physiological condition and nutritional state as compared with others sampled at the same location. In regard to the samples taken at the spawning grounds, no attempt was made to differentiate between fish which had just spawned and ones for which a time lapse had occurred after completion of spawning.

In spite of the variation in liver glycogen between individual fish of the same sex sampled at the same location, an over-all trend was seen in relation to the

levels of glycogen in the two sexes. The most striking feature of the data in Table I is the large and consistent difference between both sexes at all three points. The mean glycogen level of the male liver was 8 times more at Albion, 3.7 times more at Siwash Bridge, and 7.3 times more at the spawning grounds than that of the female.

Whereas the liver glycogen of the male decreased 61.8% from Albion to Siwash Bridge, the loss from the female was only 16%. From Siwash Bridge to the spawning grounds these levels increased so that values in postspawned fish were 1.8 times higher for the male and 1.98 times higher for the female than those found at Albion.

Unfortunately, the samples obtained from "prespawned" salmon on the Chilcotin River experienced a short period of thawing before they were analyzed (the dry ice evaporated during the night and the temperature outside the box was +3° C). Consequently, the data for these samples have been presented as minimal values only and were included to indicate the appreciably higher levels which may have been contained in these samples since a loss in liver glycogen was found in the Albion samples which had undergone the same period of thawing when compared with samples that had been analyzed prior to being thawed. Table II shows the effect of thawing on the Albion samples.

TABLE II

Glycogen content of Albion sockeye livers before and after thawing

Sex	Before, mg/g*	After, mg/g*	Decrease due to thawing, %†
M	22.17	14.25	35.7
M	11.99	3.74	68.9
M	2.73	0.40	85.4
F	0.517	None	100
F	3.69	0.31	91.6

*Based on the wet weight of the liver.
†These samples were analyzed prior to thawing and stored in the same box with the samples from prespawned fish which were analyzed after thawing.

Discussion

The liver glycogen values, and the variation for individual fish obtained in this study, are similar to those reported by Fontaine and Hatey (5) for the Atlantic salmon, *Salmo salar*, with respect to the fish captured during the winter at the spawning grounds. On the other hand, Greene (6) found lower liver glycogen levels in the king salmon, *Oncorhynchus tschawytscha*, whose average values at the beginning and end of migration were only 0.405% and 0.057% respectively. Another difference was that a decrease occurred in the liver glycogen content of the king salmon during reproductive migration, but an increase in content was found in the Chilko Lake sockeye migrant.

It is of interest to note that the liver glycogen content of males at the Chilcotin River spawning grounds (4.5%) fell within the range found for normal human liver (0.31–5.98% with a mean of 2.2%) (7) and compared with that of the rat (2.5–8.3%) and dog (6.1%) (8).

The sex difference in hepatic glycogen content in normal fasting and pregnant mammals has been described (9, 10, 11, 12). This inequality of liver glycogen in the two sexes was found during spawning migration of *Salmo salar* (5) and the observations of these workers have been confirmed in the present study. The smaller glycogen deposition in the liver of the female as compared to that in the male may be associated with the greater increase in the weight of the ovaries (1) and the accumulation of carbohydrates in the ovaries. A glucose content of 0.096% (6) and a glycogen content of 0.119% (5) have been reported for roe. Fontaine and Hatey (5) found that the glycogen content of the testes of the Atlantic salmon was only 0.017%. Furthermore, the smaller amount of liver glycogen in the female may be related to a greater estrogenic hormone level produced during the rapid sexual development associated with spawning migration. The depressant effect on liver glycogen levels following administration of natural estrogens has been demonstrated with male rabbits (13) and with rats (10). This effect of a hypersecretion of hormones, associated with the reproductive cycle, on liver glycogen deposition during spawning migration is suggested when the results obtained on *Salmo salar* (5), captured in the spring at the beginning of river migration, are compared to those of the same fish at the spawning grounds in the winter. The spawning migration of *Salmo salar* takes place over a period of many months and involves a slow and gradual development of the gonads. On the other hand, Chilko Lake sockeye spend only 36 days between Albion and the completion of the spawning act (14). Therefore, the results obtained by Fontaine and Hatey at the beginning of river migration have no chronological equivalent for most Pacific sockeye since the sockeye would be feeding before its entrance into fresh water. A run of sockeye to Cultus Lake, B.C., probably approximates most the long residence of *Salmo salar* in fresh water.[2]

Although the average liver glycogen value for the postspawning female on the Chilcotin River was 1.98 times greater than that at Albion, the variations in the individual samples were too great for the mean values to be very significant ($p > .05$). This increase was statistically significant for the male. These results may be interpreted in relation to energy expenditure studies which were carried out on the 1956 Chilko Lake run. In these considerations the two locations, Siwash Bridge and Keighley Holes, are only 18 miles apart and may be regarded as comparable points. It takes the fish 17 days to travel from Albion to Siwash Bridge and spawning begins 16–17 days later. Spawning is completed in 2–3 days.

From the fat and protein expenditure studies (14), in terms of kilogram-calories expended per day of travel, the standard male fish used 68.4 Cal/day of fat and 0.313 Cal/day of body protein in travelling from Albion to Keighley Holes, while the standard female fish consumed 87.5 Cal/day of fat and 10.1 Cal/day of protein for the same distance. From Keighley Holes to the completion of spawning the amount of fat expended by the standard male was 67.3 Cal/day and for the standard female was 68.5 Cal/day. At the same time,

[2]Personal communication from S. R. Killick of the International Pacific Salmon Fisheries Commission.

rotein consumption increased in both sexes, with the standard male using 7.7 Cal/day and the standard female 31.8 Cal/day. Thus, fat was the principal ource of energy between Albion and Keighley Holes (Siwash Bridge) and dur- ig this period there was a decrease in liver glycogen. From Siwash Bridge to 'hilko Lake a change from predominantly fat metabolism to an increased rotein metabolism was coincident with an increase in liver glycogen between nese two points. This finding is consistent with that for normal fasting animals 15) where an initial decrease is followed by a rise in liver glycogen which is ccompanied by a parallel increase in urinary nitrogen excretion of a magnitude ufficient to account for the extra carbohydrate in terms of increased protein atabolism.

Although gluconeogenesis from non-carbohydrate sources occurs in normal asted animals to replenish the depleted liver glycogen somewhat, this level is sually far below that encountered in the non-fasting state (15). However, with jections of cortical extracts, and especially those with the oxygen function at -11 and C-17 (16, 17), liver glycogen levels during fasting are usually increased o equal or to approach those of the non-fasting animals. It has been reported hat the adrenal cortical steroids do not influence the rate of glucose utilization ut increase the production of glucose by increased gluconeogenesis. Rosen *t al.* (18) have found that glucocorticoids markedly stimulate in liver the ctivity of glutamic–pyruvic transaminase, which they propose as the enzyma- ic basis in gluconeogenesis. Recently, cortisone and cortisol were found in igh concentration in the plasma of migrating sockeye salmon, and the levels t the spawning ground were much greater than those at the beginning of nigration (4). Therefore, the appearance of elevated cortical steroid levels ogether with the metabolism of body protein may be regarded as factors which ontribute to the increased levels in liver glycogen as the fish approach the spawning grounds.

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LINGCOD MUSCLE PHOSPHOMONOESTERASES

I. ACID PHOSPHATASES THAT HYDROLYZE *p*-NITROPHENYL PHOSPHATE[1]

NEIL TOMLINSON AND R. A. J. WARREN

Abstract

Five fractions (A to E), each possessing acid phosphomonoesterase activity, were separated from an aqueous extract of the muscle of lingcod (*Ophiodon elongatus*) by stepwise chromatography on diethylaminoethyl cellulose in the free-base form.

Fraction A required Zn^{++} or Mn^{++} for activity, was inhibited by heparin, and had its pH optimum at 6.0. Fraction E required Zn^{++} for activity, was not inhibited by heparin, and had its pH optimum at 5.5. Fractions B, C, and D did not require metal ions for activity, and were distinguished from each other by differences in response to pH, cysteine, ethylenediaminetetraacetate, fluoride, and tartrate.

The pH range over which fraction A was active was shifted to slightly higher values when Mn^{++} was the activator rather than Zn^{++}. Also, A was inhibited strongly by cysteine when activated by Zn^{++}, but not when activated by Mn^{++}. Data are presented that indicate these differences were due to different properties of the activating ions, rather than to the presence in fraction A of two enzymes, one activated by Zn^{++} and the other by Mn^{++}.

Introduction

Lingcod muscle contains a relatively large quantity of ribomononucleotides and other acid-soluble phosphorylated compounds (1, 2). In sterile flesh, post mortem, between 75% and 90% of the phosphorus of these compounds is released as orthophosphate. The process proceeds rapidly at room temperature and is complete in about 1 day. At 0° C, 1 to 2 weeks are required for completion (3).

Very little information is available regarding the enzymes that presumably catalyze the release of organically bound phosphorus in fish muscle post mortem (4). The occurrence of both acid and alkaline phosphatases in fish muscle has been reported (5, 6, 7), and some properties of fish muscle adenosinetriphosphatase have been described (8, 9, 10); but apart from this, no other information regarding the phosphatases of fish muscle appears to be available. An investigation of such enzymes from lingcod muscle has been undertaken, and the present paper describes the separation and some properties of five acid phosphomonoesterases from this source.

Materials and Methods

Enzyme solutions suitable for chromatography on diethylaminoethyl cellulose (DEAE)* were prepared from aqueous extracts of lingcod muscle by ammonium sulphate precipitation, followed by dialysis and lyophilization as previously described (11). The solution must be practically free from sulphate before chromatography is undertaken. In the following report an extract prepared in this fashion is referred to as a crude preparation.

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*Abbreviations: DEAE, diethylaminoethyl cellulose; EDTA, ethylenediaminetetraacetate; Tris, tris(hydroxymethyl)aminomethane.

Phosphomonoesterase activity was measured by determination of *p*-nitrophenol released from disodium *p*-nitrophenyl phosphate (Sigma Chemical Co.). The reaction mixture contained sodium succinate buffer of appropriate pH, 0.025 *M*, substrate 0.018 *M*, other additions as indicated, and enzyme. Controls with enzyme omitted were used. The incubation temperature was 25° C. Aliquots (0.33 ml) of the reaction mixture were removed immediately after the addition of the enzyme and again after a suitable period of incubation. These were at once mixed with 1 ml of 0.1 *N* NaOH, and the optical densities of the solutions were measured at 400 mμ. The quantity of *p*-nitrophenol formed was determined by reference to a calibration curve. When certain salts (e.g., those of manganese) were added to the reaction mixture, the precipitate which formed on addition of the alkali was removed by centrifugation before the light transmission measurement was made.

The chromatography of protein on DEAE in the free-base form, and the procedures for protein and light absorption measurements, have been previously described (12).

Results and Discussion

Preliminary observations indicated the presence of at least four acid phosphomonoesterases in the crude lingcod muscle preparations. A portion of the phosphomonoesterase activity of the crude preparation was very labile at room temperature in the absence of substrate at pH 5.5 or lower. The loss of this activity was accompanied by a marked alteration in the shape of the pH response curve. The crude preparation had a pH optimum at 5.3, whereas after the labile activity had been destroyed by adjusting the preparation to pH 4.5 and holding it for 1 hour at 25° C, the resulting preparation had a broad pH optimum between pH 3.5 and 5.0. This treatment caused the loss of over 70% of the activity of the crude preparation measured at pH 5.3, but only a very slight loss of activity at pH 3.5. These observations suggested that at least two acid phosphatases were present. Further, zinc ions were found to increase the activity of the crude preparation at values above 4.4, but to be inhibitory at pH 4.4 and lower. With zinc ions added the crude preparation and the preparation treated at pH 4.5, both exhibited the same pH optimum of 6.0. When the crude preparation was heated at 58° C for 10 minutes it appeared to lose all phosphatase activity. However, a portion of the activity could be restored by the addition of zinc ions to the preparation, and it then exhibited a pH optimum at 5.5. These additional observations appeared to be most readily explained if the presence of two more phosphomonoesterases, each activated by zinc, was assumed. In order to determine whether or not these explanations were correct, a chromatographic technique that had proved useful in the separation of other enzymes from lingcod muscle (12) was used to examine a crude preparation. The results are presented in Fig. 1.

It was found to be possible to separate the preparation into five enzymatically active fractions. These are designated A to E in the figure in accordance with the order of their elution from the column. Since a single component can be eluted in consecutive steps in the stepwise chromatographic technique (13), it

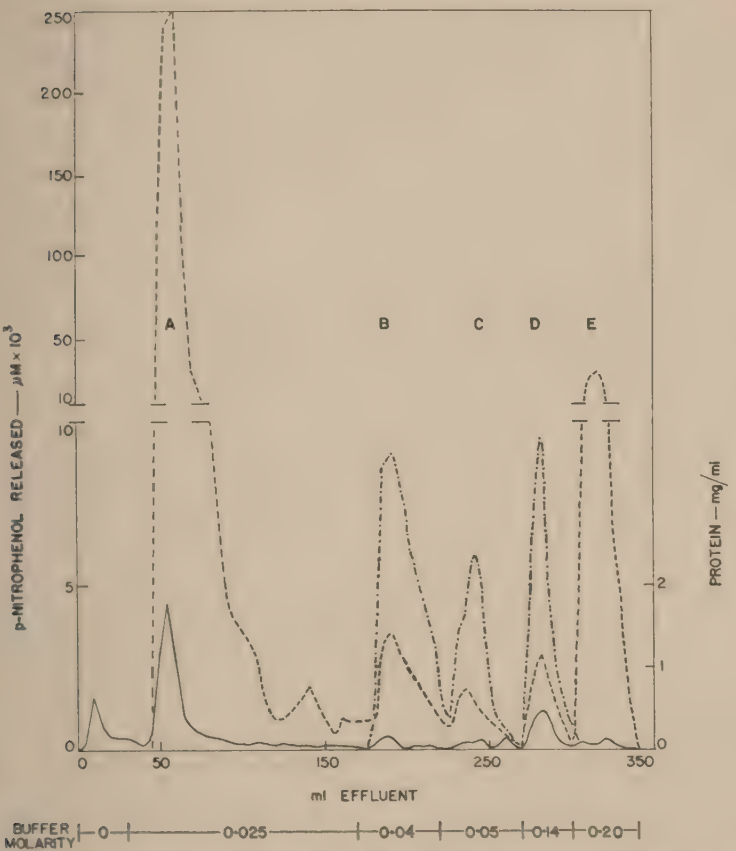

FIG. 1. Chromatographic separation of acid phosphomonoesterases from lingcod muscle. The column was 2.5 cm long by 18 mm diameter and contained 0.7 g DEAE in the free-base form. Protein applied, 63 mg. Stepwise elution, following distilled water wash, with Tris . HCl, pH 7.0 at concentrations indicated. Flow rate, 1 ml/min. Fractions, 5 ml. First fraction collected during addition of protein. Protein ———. Phosphomonoesterase activity assayed without zinc added – · – · –, with zinc added — — —. Enzyme assay system contained 0.1 ml of the fraction assayed/0.33 ml of reaction mixture, buffer pH 5.5, and $ZnSO_4$ (where indicated) 0.005 *M*. Incubation time 2 hours.

was necessary to show that the fractions had different properties before it could be concluded that they contained distinct phosphomonoesterases. In Tables I and II data are presented that support the view that each of the five fractions did indeed contain a distinct enzyme. The most marked points of difference between the fractions may be summarized as follows:

1. The influence of pH on each of the five fractions was different.
2. Fractions A and E required the addition of a metal ion for activity (see below), whereas B, C, and D did not.
3. Fractions A and E were inhibited by EDTA, whereas B, C, and D were either unaffected or stimulated by this compound.

TABLE I

The influence of pH on the phosphomonoesterase activity of chromatographically separated fractions from lingcod muscle

Fraction*	pH range for activity†	Optimum pH
A (Zn)	4.7 to 8.2	6.0
A (Mn)	5.0 to above 8.2‡	6.0
B	3.0 to 7.0	3.5 to 5.0 (broad)
C	3.0 to 7.0	5.0
D	3.0 to 7.5	5.5
E (Zn)	4.4 to 8.0	5.5

*Fractions A and E required the addition of metal ions for activity. $ZnSO_4$ or $MnSO_4$, 0.005 *M*, added as indicated.
†Buffers used: glycine. HCl, pH 2.5 to 3.6; Na succinate, pH 3.7 to 7.2; Tris . HCl, pH 7.1 to 8.2.
‡At pH 8.2 fraction A, when activated by manganous ion, exhibited about 30% of the activity shown at the optimum pH. The upper limit of pH for activity was not determined.

TABLE II

The influence of potential stimulators or inhibitors on the phosphomonoesterase activity of chromatographically separated fractions from lingcod muscle

		Fraction*					
		A(Zn)	A(Mn)	B	C	D	E(Zn)
Addition	Conc.	Activity (% of control)†					
None (control)		100	100	100	100	100	100
Cysteine	0.005 *M*	3	108	141	280	173	52
EDTA	0.022 *M*	4	0	98	279	138	1
Formaldehyde	0.17 *M*	15	5	70	36	79	8
Na fluoride	0.5 m*M*	104	103	0	46	24	102
Na fluoride	0.05 *M*	98	60	0	39	11	94
Na tartrate	0.005 *M*	52	70	1	97	21	81
Heparin	155 μg/ml	14	39	65	44	60	106

*Fractions A and E required the addition of a metal ion for activity. $ZnSO_4$ or $MnSO_4$, 0.005 *M*, added as indicated.
†Experimental conditions: Protein/0.33 ml of reaction mixture: fraction A, 50 μg; B, 6 μg; C, 3 μg; D, 22 μg; E, 4 μg. Period of incubation, 1 hour. Buffer pH 5.5. Since the activity of the enzymes, following chromatography, was rather labile in the absence of substrate at pH 5.5, they were the last component to be added to the reaction mixture.

4. Fractions A and E were more sensitive to inhibition by formaldehyde than were B, C, and D, but B, C, and D were much more sensitive to fluoride than were A and E.
5. Fraction A was activated by either zinc or manganous ions, but E was activated only by zinc (see below). Fraction A was inhibited by heparin, while E was not.
6. Fraction B was much more sensitive to inhibition by fluoride and tartrate than was C, but was only slightly affected by cysteine or EDTA, whereas C was strongly stimulated by these two compounds. Fraction D was intermediate between B and C in its sensitivity to these compounds, but tended to resemble B more closely than C.

As will be noted in Fig. 1, fractions A and E failed to show any phosphomonoesterase activity in the absence of added metal ions. The ability of various salts (each at a concentration of 0.005 *M*) to activate fractions A and E was investigated. $CaCl_2$, $CdSO_4$, $CoSO_4$, $CaSO_4$, $FeSO_4$, KCl, $NiSO_4$, $MgSO_4$, NaCl, and Na_2SO_4 did not activate either fraction. $ZnSO_4$ activated both fractions,

while $MnSO_4$ activated fraction A but not E. In Fig. 2 the course of the reaction catalyzed by fraction A in the presence of various concentrations of $ZnSO_4$ is shown. In the experiment described by this figure, the reaction was started by

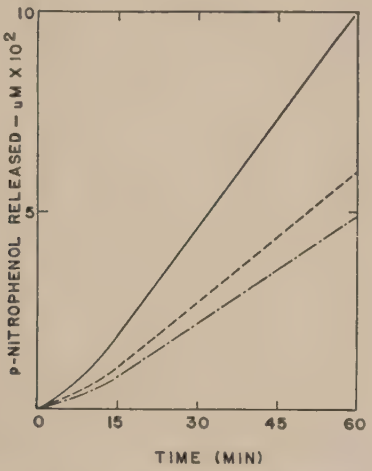

FIG. 2. The time course of the reaction catalyzed by fraction A activated by various concentrations of $ZnSO_4$. Protein, 53 μg/0.33 ml of reaction mixture. Buffer pH 5.5. Reaction started by the addition of protein. $ZnSO_4$ 0.00125 *M* —·—·—, 0.005 *M* ———, 0.02 *M* — — —.

adding the enzyme to the otherwise complete mixture after a 15-minute period of temperature equilibration. Under these conditions, after the addition of the enzyme there was a gradual increase in the rate of the reaction for 10 to 15 minutes, after which the rate became constant. When the order of addition was altered so that either $ZnSO_4$ or substrate was the last component added to the mixture, the period of increasing rate was shortened to less than 5 minutes. The same course of reaction was followed at all concentrations of $ZnSO_4$ tested. Similar results were obtained with $MnSO_4$ as activator of fraction A, or with $ZnSO_4$ and fraction E. In Fig. 3 the influence of different concentrations of $ZnSO_4$ and $MnSO_4$ on the activity of the fractions is shown. The optimum concentrations of $ZnSO_4$ and $MnSO_4$ for A were about 0.007 *M* and 0.01 *M*, respectively, while for E the optimum concentration of $ZnSO_4$ was about 0.016 *M*.

The data of Table I indicate that the pH range over which fraction A showed activity was displaced a little toward the higher pH's when $MnSO_4$ was the activator rather than $ZnSO_4$, and the data of Table II show that the effects of some possible stimulators or inhibitors (14, 15, 16, 17) on the activity of this fraction varied depending on whether $ZnSO_4$ or $MnSO_4$ was the activator. At first glance these facts might be interpreted as indicating the possible presence of two different enzymes in fraction A, one activated by zinc ions, the other by manganous ions. However, the small shift in pH range for activity, and the ability of cysteine to inhibit when zinc but not when manganese was the activator, appear to be related to properties of the activating ions. Thus, when

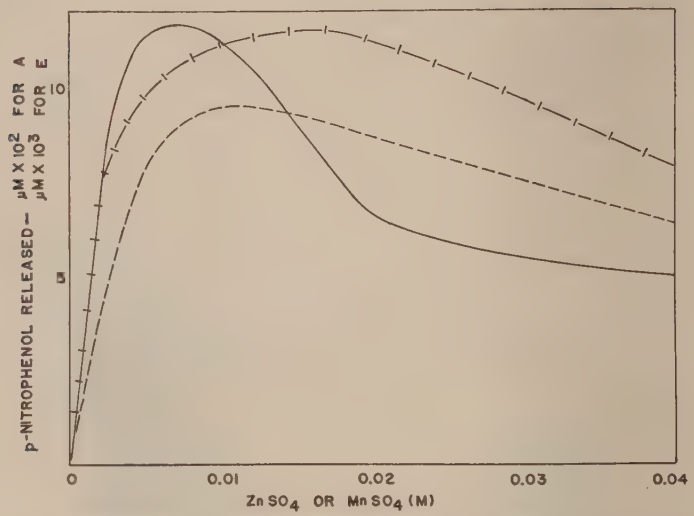

FIG. 3. The influence of various concentrations of $ZnSO_4$ and $MnSO_4$ on the activity of fractions A and E. Protein: fraction A, 65 μg/0.33 ml of reaction mixture; fraction E, 2 μg. Incubation time 2 hours. Buffer pH 5.5. Fraction A with $ZnSO_4$ ———, A with $MnSO_4$ — — —, E with $ZnSO_4$ —|—|.

the ability of cysteine to complex with zinc and manganous ions was investigated by means of the procedure of Martin and Edsall (18), it was found that cysteine formed a complex with zinc ion at pH 4.3 and higher, but not with manganous ion below pH 7.7. Togasawa (19) has presented spectrophotometric evidence that manganese and cysteine do not complex below pH 8.0. As the effect of cysteine on the activity of the fraction was studied at pH 5.5 the observed results could be expected even if only one enzyme was involved. Again, with regard to the small shift in the pH range for activity of the fraction, manganous hydroxide is very much more soluble than is zinc hydroxide, and this fact will account for the effect observed at the upper end of the range, while the small difference at the lower end probably reflects a difference in the ability of the two ions to complex with the enzyme. The other differences observed are quantitatively smaller, but no explanation for them can be offered — they may or may not be related to properties of the activating ions. However, direct evidence that two enzymes, one activated by zinc and the other by manganese, were not responsible for the activity of fraction A was provided (1) by the fact that when the chromatographic fractions making up fraction A in Fig. 1 were assayed with manganese as activator, rather than zinc, the results exactly paralleled those obtained with zinc as activator, thus showing that there was no tendency for the activities to separate on the column, and (2) by the data of Table III. These show that when the two ions were used together at nearly their optimum concentrations (0.005 *M* and 0.01 *M* for zinc and manganese, respectively) to activate an amount of enzyme that would limit the rate of the reaction with either of the ions separately, then their activating effects were not additive. In view of the fact that their effects were additive when

TABLE III

The influence of $MnSO_4$ and $ZnSO_4$ separately and together on the phosphomonoesterase activity of fraction A from lingcod muscle

Addition		*p*-Nitrophenol released,*
$ZnSO_4$	$MnSO_4$	$\mu M \times 10^3$
0.00125 *M*	—	14.0
—	0.0025 *M*	10.8
0.00125 *M*	0.0025 *M*	26.0
0.005 *M*	—	22.0
—	0.01 *M*	18.4
0.005 *M*	0.01 *M*	24.8

*Experimental conditions: Protein/0.33 ml of reaction mixture, 12 μg. Incubation time 30 minutes. Buffer pH 6.0.

they acted together at less than their optimum concentrations (0.00125 *M* and 0.0025 *M*), thus apparently ruling out the possibility that if two separate enzymes were involved, then the ion activating one might inhibit the other, this strongly suggests that each of the ions was not activating a separate enzyme.

The small peak of activity observed in the trailing edge of the peak of fraction A (at about 140 ml of effluent in Fig. 1) was not reproducible and did not always appear even when the same crude preparation was chromatographed on separate occasions. It is regarded as an artifact, although no explanation for its appearance can be given. The enzymatic activity of fractions A and E was completely destroyed by heating them at 100° C for 1 minute.

In Table IV the influence of various salts (each at a concentration of 0.005 *M*) on the phosphomonoesterase activity of fractions B, C, and D is recorded. The activity of the fractions was inhibited by nearly all the salts tested—even

TABLE IV

The influence of salts on the phosphomonoesterase activity of fractions B, C, and D from lingcod muscle

	Fraction		
	A	B	C
Addition*	Activity (% of control)		
None (control)	100	100	100
$CaCl_2$	98	76	91
$CdSO_4$	44	22	26
$CoSO_4$	32	5	24
$CuSO_4$	0	3	2
$FeSO_4$	50	36	61
KCl	100	67	77
$MgSO_4$	75	52	80
$MnSO_4$	97	109	112
Na_2SO_4	94	79	99
$NiSO_4$	0	8	16
$ZnSO_4$	41	50	50

*All salts added at a concentration of 0.005 *M*. Other conditions as in Table II.

KCl and Na_2SO_4 caused some inhibition—but $MnSO_4$ stimulated the activity of fractions C and D to a small extent. Although it is not shown in the table, some additional observations that appear to be of interest were made of the effects of certain combinations of inhibitors and stimulators on the activity of fractions B, C, and D. When cysteine and $MnSO_4$ (each 0.005 *M*) were added together, their separate effects on each of the fractions were found to be additive. When EDTA and sodium fluoride (the latter at 0.5 mmolar) were added together, the inhibitory effect of fluoride on fractions B and D was almost completely prevented, but fluoride inhibition of C was not prevented. When EDTA, or cysteine, and tartrate were added together, the effect of tartrate on each fraction was not altered.

In summary, the evidence clearly indicates that fraction B corresponds to the phosphomonoesterase activity in the crude preparation resistant to destruction at pH 4.5 in the absence of substrate, while C and D together correspond to the activity labile under these conditions. Fraction A corresponds to the activity of the crude preparation activated by zinc ions, but labile to heating at 58° C at pH 7.0, and fraction E to the activity activated by zinc ions and resistant to heating at 58° C at pH 7.0. There are thus a minimum of five acid phosphomonoesterases in lingcod muscle each able to hydrolyze *p*-nitrophenyl phosphate.

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THE ENZYME 5-PHOSPHORYLRIBOSE PYROPHOSPHOKINASE IN FISH MUSCLE[1]

H. L. A. Tarr

Abstract

The enzyme 5-phosphorylribose pyrophosphokinase was found in the muscles, and in certain of the organs, of marine teleost fish. It was considerably purified at between 0 and $-1°$ C by a simple, rapid procedure which involved extraction of the muscle with water, precipitation of the active fraction at pH 5.5, re-solution of the precipitate in 0.1 *M* phosphate buffer pH 7.0, and removal of about half the inactive protein from the solution by treatment with alumina C_γ gel. The enzyme was found to be very unstable, purified preparations losing activity quite rapidly even when stored at less than $-50°$ C. The requirement of the enzyme for ATP, R5P, Mg^{++}, and glutathione, and its pH–activity relationship, were investigated. The lithium salt of PRPP was prepared in good yield and in a comparatively high state of purity using the purified muscle enzyme from lingcod.

Introduction

Kornberg *et al.* (1) were the first to report the isolation of PRPP and they subsequently gave a more detailed account of its enzymic synthesis from ATP and R5P by a pigeon liver enzyme (Kornberg *et al.* (2)). In independent studies which were largely concerned with the biosynthesis of purines, Korn *et al.* (3) and Remy *et al.* (4) isolated the same compound and showed that the pyrophosphate group was in the α position. Tener and Khorana (5) achieved chemical synthesis of the compound as the lithium salt, though in rather poor yield. They produced unequivocal evidence that the position of the pyrophosphate group was α. Khorana *et al.* (6) showed that the enzymic synthesis of PRPP involved a direct transfer of the terminal pyrophosphate group of ATP to carbon 1 of R5P. The pivotal role which PRPP plays in biosynthesis of purine and pyrimidine nucleotides was reviewed by Carter (7) and more recently by Hartman and Buchanan (8). The last-named investigators described the complex series of enzymic processes which are involved in the *de novo* synthesis of inosinic acid from R5P and ATP via PRPP and other intermediates.

Materials and Methods

In preliminary studies a sample of lithium PRPP kindly supplied by Dr. Gordon Tener of the B.C. Research Council was used for reference. In the initial stages of the investigation the dried yeast used to prepare the "alcohol fraction" containing orotodine 5′-phosphate pyrophosphorylase and carboxylase enzymes used in the enzymic assay of PRPP (Lieberman *et al.* (9)) was that which had been used by Dr. A. Kornberg. This yeast, which was generously donated by Dr. Gobind Khorana of the B.C. Research Council, had been stored

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The following abbreviations are used: PRPP, 5-phosphoryl α-D-ribofuranose 1-pyrophosphate; R5P, D-ribose 5-phosphate; DR5P, 2-D-deoxyribose 5-phosphate; AMP, adenosine 5′ phosphate; ADP, adenosinediphosphate; ATP, adenosinetriphosphate; Tris, trishydroxymethylaminomethane; PRPPkinase, 5-phosphorylribose pyrophosphokinase.

frozen for several years and had lost much of its original activity. It was found subsequently that a water-washed, air-dried "starter" yeast, kindly supplied by Mr. J. Neilson of the Vancouver branch of Carling Breweries, Ltd., gave an enzyme preparation with activity practically identical with that described by Lieberman *et al.* (9). The dried yeast and the enzyme preparations made from it were stored over dry ice (less than $-50°$ C) during the course of the work.

Barium deoxyribose 5-phosphate was prepared as described by Kornberg *et al.* (2). It was about 95% pure as determined by its content of deoxyribose and phosphorus and the fact that almost complete hydrolysis of the orthophosphate group occurred in 30 minutes in 1 *N* HCl at 100° C (Racker (10)). Barium ribose 5-phosphate was prepared by the method of Khym *et al.* (11). Aqueous solutions of these compounds were converted to the sodium salts before use by treatment with Dowex 50 × 8 Na^+ resin. Disodium ATP and ADP were purchased from the Pabst Laboratories, Milwaukee, Wisconsin. Reduced glutathione and D-ribose were obtained from the Nutritional Biochemical Corporation, Cleveland, Ohio, and AMP from Schwarz Laboratories, Mt. Vernon, New York. Aged alumina gel Cγ (40 mg dry weight per ml) was kindly donated by Dr. W. E. Razzell of the B.C. Research Council.

Ribose was determined by the Mejbaum (12) method with 40-minute heating and D-ribose as standard. Orthophosphate in the presence of labile phosphate was determined by the method of Lowry and Lopez (13) with the modification suggested by Bruemmer and O'Dell (14). Total phosphorus was determined by the Gomori (15) procedure after hydrolysis (Umbreit *et al.* (16)). Protein was determined by the Kingsley (17) procedure. PRPP was determined by the assay B, stage II, procedure of Kornberg *et al.* (2). With the cruder enzyme preparations, PRPP formation was stopped and protein removed before assay by immersing the glass tubes containing the reaction mixtures for 40 seconds in a boiling water bath, promptly chilling the solutions in a dry-ice–alcohol bath, and centrifuging them. In experiments with purified enzyme preparations it was found that direct assay of the extracts was usually satisfactory since PRPP formation was comparatively slow at 30° C.

Experimental

With fish obtained at sea the fisherman placed samples of freshly excised muscle in polyethylene bags and froze them in contact with dry ice. Live lingcod (*Ophiodon elongatus*) were supplied through the courtesy of Mr. Murray Newman, Curator of the Vancouver Aquarium. The fish were stunned, the muscle and organs promptly excised, frozen in liquid nitrogen, and, unless otherwise stated, stored at below $-50°$ C in polyethylene bags.

In preliminary studies the distribution of PRPPkinase in different tissues or organs of fish was determined by blending a portion with 2 volumes of water at 0° C for about half a minute and centrifuging at 10,000 *g* for 5 minutes. The clear extracts were tested by adjusting them to between pH 7.4 and 7.5 and incubating 0.33 ml in a total volume of 1.0 ml with ATP and R5P exactly as in the assay B, stage I, procedure of Kornberg *et al.* (2). PRPP formation was normally linear for up to 6 hours at 30° C under these conditions. This assay

was adopted since it was the only one known which was available for studying PRPP formation. Toward the end of the investigation it was found that the ATP and GSH concentrations as specified in this procedure were somewhat suboptimal for the fish muscle enzyme, but it was felt that the differences did not warrant a complete reinvestigation using more optimal concentrations of these compounds.

The results of tests with a number of aqueous tissue extracts (Table I) indicated that, apart from spleen and milt, only the muscle showed considerable activity. The lability of the enzyme system in the muscle and crude extracts is

TABLE I

Formation of PRPP by aqueous extracts of fish tissues

Source*	Treatment	Protein (mg/ml) of extract	Specific activity† ($\times 10^3$)
Lingcod muscle[1]	Muscle stored 10 days at −25° C	9.7	15.6
Lingcod muscle[1]	Aqueous extract stored 3 days at −25° C	9.7	13.8
Lingcod muscle[2]	Fresh muscle stored 2 hours at 0° C	10.2	14.4
Lingcod muscle[2]	Frozen muscle stored 24 hours at −25° C	10.2	13.5
Lingcod muscle[2]	Frozen muscle stored 34 days at −25° C	10.2	12.3
Lingcod muscle[2]	Frozen muscle stored 42 days at −25° C	10.2	7.3
Lingcod muscle[2]	Aqueous extract freeze dried, redissolved	10.2	13.8
Lingcod muscle[3]	Fresh muscle stored 2 hours at 0° C	10.3	12.1
Lingcod muscle[3]	Aqueous extract stored 2 days at less than −50° C	10.3	11.6
Lingcod muscle[3]	Aqueous extract stored 2 days at −25° C	10.3	9.3
Lingcod muscle[3]	Aqueous extract stored 1 day at 1° C	10.3	7.8
Lingcod muscle[3]	Aqueous extract stored 4 days at 1° C	10.3	2.6
Lingcod muscle[3]	Aqueous extract adjusted to pH 9.0 and promptly readjusted to pH 7.1 at 0° C	10.3	8.1
Lingcod muscle[4]	Frozen at sea in dry ice	10.6	9.1
Lingcod muscle[5]	Frozen at sea in dry ice	10.3	8.5
Lingcod muscle[6]	Frozen at sea in dry ice	11.1	5.4
Coho salmon muscle	Frozen at sea in dry ice	9.8	8.3
Spring salmon muscle[1]	Frozen at sea in dry ice	10.0	9.0
Spring salmon muscle[2]	Frozen at sea in dry ice	10.4	7.3
Blue perch	Frozen at sea in dry ice	9.7	7.5
Lingcod spleen[3]	Stored 18 days at less than −50° C	33.0	3.0
Lingcod liver[3]	Stored 18 days at less than −50° C	40.0	0.22
Lingcod heart[3]	Stored 18 days at less than −50° C	33.5	0.36
Lingcod milt[3]	Stored 18 days at less than −50° C	8.0	2.7

*Numbers indicate different fish. All fish muscles or organs were excised from stunned fish and promptly frozen by immersion in liquid nitrogen unless otherwise specified.

†Micromoles of PRPP formed by 1 mg of protein in 1 hour at 30° C under the conditions of the standard assay (stage I).

apparent since the activity of preparations obtained by freezing the muscle in dry ice at sea was invariably lower than that obtained with muscle frozen promptly in liquid nitrogen. Also storage of the muscle, or of aqueous extracts prepared from it, at −25° C caused slow loss in activity, while in extracts held at 1° C inactivation was extremely rapid. Brief exposure to pH 9.0 at 0° C resulted in marked loss of activity. The crude muscle extracts lost little activity when partially concentrated by freeze-drying.

Preparation of Enzyme

The lability of the enzyme rather limited available purification techniques. Dialyzed ammonium sulphate precipitates made at pH 7.0 and 0° C possessed

no PRPPkinase activity. Likewise, adsorption of crude lyophilized active muscle extracts on diethylaminoethyl cellulose columns followed by gradient elution at low temperatures as used in previous work (Tarr (18)) yielded inactive protein fractions. The following rapid method resulted in considerable purification with little loss in the total activity. All steps were carried out at 0 to $-1°$ C as rapidly as possible.

Frozen lingcod muscle was shaved off, mixed well with an equal volume of water in centrifuge tubes, and centrifuged at 10,000 *g* for 10 minutes. The extraction was repeated and the pooled supernatant liquids filtered through a thin layer of glass wool and adjusted cautiously to pH 5.5 with 1 *N* acetic acid. The precipitate was collected by centrifuging 3 minutes at 10,000 *g*, the supernatant liquid drained off, and the precipitate dissolved in 0.1 *M* sodium phosphate buffer, pH 7.6, to give a total volume equal to about one-eighth of that of the original extract. This solution (pH about 7.0) was stored at less than $-50°$ C, since under these conditions it retained most of its original activity for 2 or 3 weeks. Prior to use, 1 volume of freshly washed alumina Cγ gel (40 mg solids per ml) was added to 4 volumes of the extract, and the mixture centrifuged 10 minutes at 10,000 *g*. This procedure removed about half the protein without significant loss of total activity. Attempts at further purification using calcium phosphate gel were not successful.

Table II gives the protocol of a typical purification which shows that the enzyme is concentrated about 30-fold on the basis of the original aqueous extract without serious loss of total activity. The muscle used had been stored

TABLE II

Preparation of 5-phosphorylribose pyrophosphokinase

Fraction	Volume (ml)	Protein (mg)	Total units*	Specific activity†
Aqueous extract of 20 g muscle	42.0	425	9.3	0.022
pH 5.5 precipitate	5.0	22.8	9.25	0.41
pH 5.5 supernatant	43.0	394	1.45	0.0036
Alumina Cγ gel supernatant	5.5	12	8.30	0.69

*Amount of enzyme forming 1 μmole of PRPP per hour at 30° C under the conditions of the standard assay (stage I) is one unit.
†Units per mg of protein.

4 months at less than $-50°$ C and had lost only a little of its original activity. On the assumption that the fish muscle contained 18% protein, purification would be about 250-fold assuming no loss of activity occurred in preparation of the aqueous extract. The specific activity of another Cγ alumina gel treated preparation was 1.0. On a protein basis the most active preparation had about 1/30 of the specific activity of the best preparations made from acetone-treated pigeon liver (Kornberg *et al.* (2)). Preparations treated with alumina Cγ gel were used soon after preparation, for even when stored at less than $-50°$ C they lost their activity in a few weeks.

pH *Activity*

Assay B, stage I, medium (subsequently referred to as the standard medium) of Kornberg *et al.* (2) was prepared without added enzyme, and portions of the

chilled solution were adjusted to the pH values indicated in Fig. 1 by addition of very small amounts of 0.025 *M* acetic acid or 0.1 *M* tris solution. Glass tubes (7.5 × 100 mm) containing 0.95 ml of buffered solution and 0.5 ml

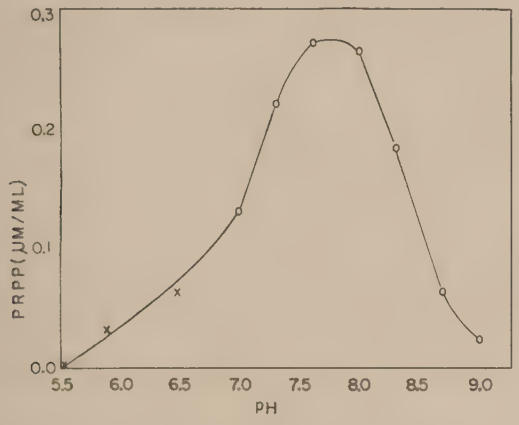

FIG. 1. The pH–activity curve for PRPPkinase. Acetate, ×——×; Tris buffers, ○——○.

(375 μg protein) of purified PRPPkinase were incubated 3 hours at 30°. PRPP formation was approximately linear at all pH values studied for this period. The tubes were immersed 40 seconds in a boiling water bath, promptly chilled in a freezing mixture of dry ice and alcohol, and centrifuged to remove precipitated protein. PRPP was determined enzymically using 0.20-ml portions in the standard assay. The results (Fig. 1) show that the kinase has a fairly sharp optimum between pH 7.5 and 8.0, is inactive at pH 5.5, and almost inactive at pH 9.0.

Effect of R5P and ATP Concentrations on PRPP Formation

Tubes containing the constituents of the standard assay medium were prepared, the concentration of R5P being varied with the ATP concentration constant at 1.8×10^{-3} *M*, and the ATP concentration varied with the R5P concentration constant at 1.25×10^{-3} *M*. Each tube received purified enzyme (300 μg protein) in a total volume of 1 ml. The amount of PRPP present was determined after 3 hours at 30° C, using 0.2-ml aliquots. The results (Fig. 2) show that a concentration of about 5×10^{-4} *M* R5P is required for maximum activity, and that concentrations in excess of this and not exceeding 5×10^{-3} *M* do not affect PRPP formation. On the other hand, PRPP synthesis is reduced when concentrations of ATP above about 2.5×10^{-3} *M* or below 1.5×10^{-3} *M* are used. No PRPP synthesis occurred when DR5P replaced R5P in the standard method, even when the concentration was increased to 5.0 μmoles ml.

Effect of Mg^{++} *Glutathione and* F^{-}

The concentrations of reduced glutathione, or of Mg^{++} (as $MgCl_2$) were varied in the standard assay medium, each tube receiving 370 μg of purified enzyme protein. The PRPP concentration was determined as usual after

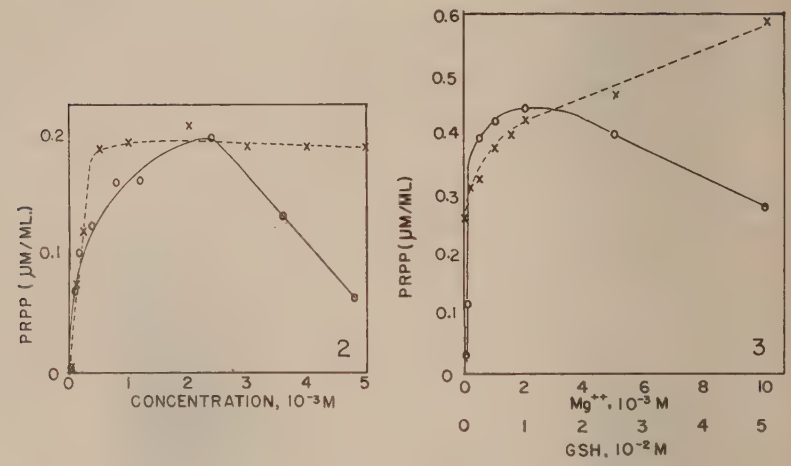

FIG. 2. Effect of ATP (○——○) and R5P (×– – –×) on PRPP formation.
FIG. 3. Effect of Mg^{++} (○——○) and GSH (×– – –×) on PRPP formation.

2 hours at 30° C. The results (Fig. 3) show that the enzyme has an absolute requirement for Mg^{++} and that the optimum concentration of this cation is about 2×10^{-3} *M*. PRPP formation proceeded in the absence of added glutathione, but its addition occasioned an increase in the amount of PRPP formed which was maximal at the highest concentration of glutathione studied (5×10^{-2} *M*). Kornberg *et al.* (2) did not indicate whether the concentration of GSH used in their assay was optimal.

In the absence of KF only about half as much PRPP was formed as when 0.01 to 0.10 *M* KF was added to the standard assay medium. However, in 0.20 *M* concentration, fluoride was inhibitory.

Balanced Studies of the Reaction

Kornberg *et al.* (2) showed that, under the conditions of the standard assay where the initial concentrations of ATP and R5P were 1.2×10^{3} *M* and 2.5×10^{-3} *M* respectively, about 90% of the ATP was converted to PRPP and AMP. In the present work, with the enzyme preparations used it has not been possible to achieve this condition. Thus, under the conditions of the standard assay (225 μg/ml enzyme protein), and commencing the reaction with 0.25, 0.50, and 1.0 μmole per ml each of R5P and ATP, only approximately 80%, 60%, and 40% respectively of the reactants could be accounted for as PRPP (Fig. 4). Even when three times the concentration of enzyme was employed with 1 μmole/ml of reactants, only 58% could be accounted for as PRPP after 5 hours, there being no further increase up to 7 hours. A slow linear decrease in PRPP concentration occurs in the complete assay medium when heat-inactivated enzyme is used, presumably due to formation of 5-phosphorylribose 1,2-cyclic phosphate. This is by no means as marked as that which occurs when PRPP and AMP are incubated with the active enzyme preparation (Fig. 4). The prompt decrease in PRPP which occurs under the above conditions has

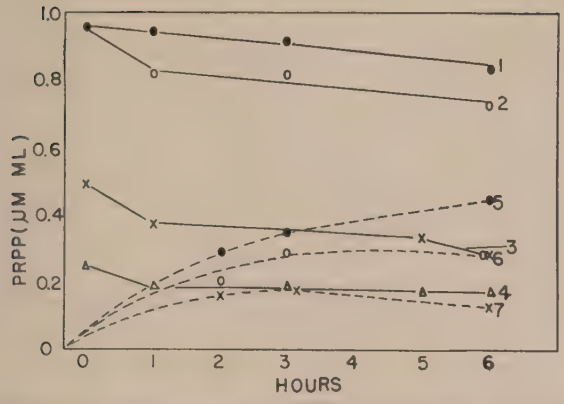

FIG. 4. Formation or disappearance of PRPP starting with ATP plus R5P or with PRPP plus AMP. Curve 1: 1 μmole each of PRPP plus AMP, heated enzyme control. Curves 2, 3, and 4: 1.0, 0.5, and 0.25 μmole/ml each of PRPP plus AMP. Curves 5, 6, and 7: 1.0, 0.5, and 0.25 μmole/ml each of R5P plus ATP. Enzyme concentration 225 μg protein per ml.

not yet been explained, but it could be at least partially accounted for by the possible presence in the enzyme preparation used of purine or pyrimidine pyrophorylase enzymes. Such enzymes could cause reaction of PRPP with small amounts of these bases, which may occur in the enzyme preparation, to form nucleotides (Kornberg *et al.* (19)).

When mixtures containing 0.5 μmoles/ml of AMP and PRPP were incubated 2 hours at 30° with purified enzyme under the above conditions, reduced to a very small volume by freeze-drying, and the concentrates submitted to descending chromatography using an isobutyric acid solvent system (Zetterström and Ljunggren (20)), no R5P or ATP could be demonstrated. With paper electrophoresis of the concentrates using Whatman No. 3 paper and 0.05 *M* sodium citrate buffer, pH 3.5 (30 v/cm/1.5 hours) (Smith (21)), a system which occasions excellent separation of ATP, ADP, and AMP, no trace of ATP was found when the appropriate area of paper was eluted and the solution examined spectroscopically at 258 mμ. This indicates that the reaction is almost certainly irreversible. A large proportion of the AMP had disappeared and a zone which corresponded with IMP had appeared in both systems, indicating the presence of a strong AMP deaminase in the enzyme preparation.

Preparation of PRPP

The general procedure of Khorana *et al.* (6) was followed. ATP, 1 mmole; R5P, 1 mmole; glutathione, 10 mmole; KF, 50 mmole; $MgCl_2$, 2 mmole; potassium phosphate buffer (pH 7.6), 20 mmole; and 50 ml of purified PRPP-kinase (188 mg protein) in a final volume of 1 liter were incubated at 30° C. After 4 hours, 430 μmoles of PRPP were present and the amount did not increase appreciably after a further 2 hours' incubation. The solution was chilled, treated with 50 g of Norit A acid-washed charcoal, which was sufficient to remove practically all substances causing absorption at 260 mμ, centrifuged at 3° C for 2 minutes at 10,000 g, and filtered by gravity through Whatman

No. 31 fluted filter paper. The charcoal treatment occasioned a considerable loss of PRPP since assays showed 328 μmoles were present at this stage.

All subsequent operations were carried out below 3° C. The solution was absorbed on a 20 × 5 (diam.) cm column of Dowex 1 × 10 (200–400 mesh) resin in the chloride form, the column washed with 1 liter of water, and elution carried out at 2 liters per hour, first with 9 liters of 0.1 *M* LiCl (pH about 6.3) to remove Pi and R5P and subsequently with 0.2 *M* LiCl. The first 360 ml of eluate was discarded, the PRPP (320 μmoles) being eluted in the next 2 liters. The clear solution was concentrated to 95 ml by freeze-drying, mixed with anhydrous methanol (38 ml) and acetone (380 ml), and the precipitated lithium salt of PRPP collected by centrifuging 2 minutes at 5000 *g*. The precipitate was suspended in 25 ml of a solution containing 1 part of methanol and 2 parts of acetone, centrifuged, resuspended in 50 ml of acetone, and the precipitate dried 5 minutes over P_2O_5 using a high vacuum pump to yield 160 mg of a white powder. This gave the following analysis, in micromoles per milligram, calculated for $C_5H_7O_{14}P_3Li_5.5H_2O$; M.W. 508.82 (Tener and Khorana (5)).

	Theoretical value	Found	% of theoretical
Total ribose	1.97	1.71	87
Total P	5.90	5.15	87
Inorganic P	0	0.063	1.07
PRPP (enzymic assay)	1.97	1.66	84.5
Ratio, organic P:ribose	3:1	3.05:1	
Organic P	5.90	5.087	86

Since 84.5% of the total ribose (87%) was accounted for as PRPP, not more than 2.5% of the ribose was present as the 1,2-cyclic ester of PRPP. The yield was 266 μmoles calculated from the enzyme assay, or 62% of the amount formed initially, most of the loss occurring during the charcoal treatment.

Discussion

Since the original work of Kornberg *et al.* (2) in which it was reported that extracts of acetone powders of livers of the chicken, rat, and mouse had very much lower PRPPkinase activities than did those of pigeon liver, the distribution of this enzyme in other mammalian tissues does not appear to have been studied. The specific activities of the best preparations of fish muscle PRPPkinase reported in the present work, though much lower than that of the most active pigeon liver preparations, are not very different from those reported by Kornberg *et al.* (2) for livers of animals other than that of the pigeon. So far as the writer is aware, the presence of this enzyme has not been reported in mammalian muscles.

The general properties of the enzyme from fish muscle so far as pH–activity relationship, magnesium requirement, optimum ATP, and R5P concentrations and inability of DR5P to replace R5P are rather similar to those reported for the pigeon liver enzyme. The enzyme was found to be present, and in about the same concentration, in the muscles of several marine teleost fish. The PRPPkinase activity of liver and heart extracts was very poor compared with muscle, while spleen extracts had lower activity.

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NUTRITION AND METABOLISM OF MARINE BACTERIA

X. ION REQUIREMENTS FOR OBTAINING AND STABILIZING ISOCITRIC DEHYDROGENASE FROM A MARINE BACTERIUM[1]

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Abstract

When cells of a marine bacterium were washed and suspended in 0.05 *M* $MgCl_2$, no isocitric dehydrogenase could be demonstrated in the extract prepared, although all other tricarboxylic acid cycle enzymes had been shown to be present. Failure to detect the enzyme was traced to the presence of halide as the sole anion in the solution used to suspend the cells. Sulphate or phosphate salts gave rise to an active enzyme. These relations applied to extracts prepared by sonic treatment, alumina grinding, and lysis by versene and lysozyme. Evidence was obtained suggesting that halides fail to prevent the denaturation of the enzyme after its release from the cells. Tested under optimum conditions, the specific activity of isocitric dehydrogenase in the extract was found to be proportional to the ionic strength of the solution used to suspend the cells (maximum activity at 0.4 to 0.5 μ). In the presence of low concentrations of an anion giving rise to an active enzyme, halide salts were found capable of contributing to these ionic strength requirements. The addition of Mg^{++} to the extract in the presence of a suitable anion at least doubled the storage life of the enzyme at 4° C.

Introduction

In the course of an investigation to determine if enzymes of the tricarboxylic cid cycle were present in a marine bacterium (1, 2), cells of the organism were outinely washed and suspended in a solution of $MgCl_2$ for the preparation of xtracts by sonic treatment. A solution of $MgCl_2$ was the medium of choice for ashing the cells since Mg^{++} prevented cell lysis (3) and the enzymes in the xtracts prepared were to be tested for their response to Na^+ and K^+. All of the nzymes of the tricarboxylic acid cycle could be shown to be present in the xtracts except isocitric dehydrogenase. Since citrate-adapted whole cells of he organism had been found to oxidize citric acid with the accumulation of arge amounts of α-ketoglutaric acid (4) it seemed likely that isocitric dehydogenase was present in the intact cells. Conditions were therefore sought for he demonstration of its presence in the cell-free extract. These studies have ndicated that rather specific inorganic requirements must be met before an ctive isocitric dehydrogenase can be demonstrated routinely and its activity naintained.

Experimental

The organism used is a species of either a *Pseudomonas* or a *Spirillum*, and as been designated B-16 in this and previous communications.

Conditions for carrying the cultures, growing the cells, and preparing cell suspensions have been described (2). After being harvested from the medium by

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Contribution from the Fisheries Research Board of Canada, Technological Station, Vancouver, B.C.

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[3]Abbreviations used: Tris, trishydroxymethylaminomethane; *G*, gravity; TPN, triphosphopyridine nucleotide; μ, ionic strength.

centrifugation, cells were resuspended in a volume of wash solution equal to half the volume of the original medium. The cells were then resuspended in and centrifuged from the appropriate salt solution twice more before final suspension. All manipulations were conducted at 4° C.

Cell-free extracts were prepared in three ways. Sonic extracts were obtained by treating cell suspensions for 5 minutes in a Raytheon 10-kc sonic oscillator. Extracts by alumina grinding were prepared by the method of McIlwain (5). A cell paste was mixed with two and one-half times its wet weight of levigated alumina and rubbed with a pestle in a chilled mortar until cell breakage occurred. Cell extracts by cell lysis were obtained by applying the procedure of Repaske (6) modified to provide conditions optimum for the lysis of this organism. The lysing system contained (in amounts/ml): Tris[3] buffer 6.25 μmoles, versene 4.3 μmoles (adjusted to pH 7), crystalline lysozyme (Worthington Biochemical Corp.) 0.05 mg, cell suspension to provide 10 mg dry weight of cells per ml of the lysing system. When an all-sulphate system was desired, Tris buffer was added as the sulphate salt and K_2SO_4 was added to bring the final ionic strength to 0.3. For an all-chloride system, buffer was added as the chloride salt and the ionic strength adjusted with KCl. The mixture was incubated for 30 minutes at room temperature and then made 0.05 *M* with respect to Mg^{++} using either $MgSO_4$ or $MgCl_2$, depending on the anion desired. Desoxyribonuclease was added at a level of 0.1 mg/ml and incubation allowed to proceed for a further 30 minutes.

All extracts were finally clarified by centrifuging for 30 minutes at 50,000 $\times$ *G* in a Spinco ultracentrifuge.

Both isocitric dehydrogenase and aconitase were measured spectrophotometrically, the former by following the reduction of TPN at 340 mμ (7), the latter by determining the rate of increase in optical density at 240 mμ (8), in both cases using *dl*-isocitrate as substrate. The compositions of the test systems used are included as footnotes to the tables.

A unit of enzyme activity was taken to be that amount of enzyme producing an optical density change of 0.001 per minute. Specific activity was calculated to be the number of enzyme units per mg of protein.

Protein was determined by the biuret procedure (9) using crystalline pepsin (Worthington Biochemical Corp.) as standard.

Results

Although cells washed and suspended in 0.05 *M* $MgCl_2$ solution (0.15 μ) failed on sonic treatment to give rise to an extract containing detectable isocitric dehydrogenase, an active enzyme resulted when 0.33 *M* K_2SO_4 (0.4 μ) was used. With extracts prepared with K_2SO_4 it was possible to show that the enzyme was TPN-linked, required Mg^{++}, and was non-specifically activated, like the aconitase of this organism, by a number of inorganic salts (2).

In an effort to determine why isocitric dehydrogenase could not be demonstrated in extracts when cells were washed with $MgCl_2$, cells from the same growth medium were divided into three parts and each part washed and suspended in either $MgCl_2$, $MgSO_4$, or K_2SO_4 solutions, all of the same ionic

rength. The extracts obtained on sonic treatment were then tested for isocitric ehydrogenase and aconitase activity under conditions previously established) be optimum for the activity of these enzymes (Table I). It is evident that

TABLE I

Isocitric dehydrogenase and aconitase activity of extracts prepared by washing and suspending cells in either $MgSO_4$, $MgCl_2$, or K_2SO_4 solutions before sonic treatment

Suspending solution			Specific activity*	
Solute	M	μ	Isocitric dehydrogenase	Aconitase
$MgCl_2$	0.05	0.15	8	377
$MgSO_4$	0.0375	0.15	1793	270
K_2SO_4	0.05	0.15	787	204

*Cuvette contents for isocitric dehydrogenase: *dl*-isocitrate (Tris salt), 20 μmoles; TPN, 1 μmole; $MgCl_2$, 50 μmoles; Tris buffer, pH 8.0, 320 μmoles. Total ionic strength in cuvette, ca.0.38. Cell extract, 0.05 to 0.1 mg protein. Total vol., 3.0 ml. Temp., 25° C.
Cuvette contents for aconitase: *dl*-isocitrate (Tris salt), 30 μmoles; Tris buffer, pH 8.0, 330 μmoles. Total ionic strength in cuvette ca. 0.42. Cell extract, 0.1 to 0.25 mg protein. Total vol., 3.0 ml. Temp., 25° C.

)oth $MgSO_4$ and K_2SO_4 solutions but not $MgCl_2$ gave rise to extracts containing appreciable isocitric dehydrogenase activity while all three preparations were ictive with respect to aconitase. This finding ruled out Mg^{++} and the ionic -trength of the suspending medium as being factors responsible for the lack of socitric dehydrogenase activity in extracts prepared from cells suspended in 0.05 M $MgCl_2$. Two other possibilities were considered, either that Cl^- was nhibitory or $SO_4^=$ stimulatory for the enzyme. The first could not be the case since only 5 μmoles of Cl^- were introduced into the reaction system with the extract while some 400 μmoles (300 from the Tris buffer and 100 from the $MgCl_2$) were present in the test system under conditions found to be optimum for demonstrating isocitric dehydrogenase activity. The second possibility was tested by washing and suspending cells in a potassium phosphate solution and comparing the isocitric dehydrogenase activity of the extract prepared with that of an extract from cells from the same growth medium washed and suspended in a K_2SO_4 solution. The results (Table II) show that an extract

TABLE II

Isocitric dehydrogenase activity of extracts from cells washed and suspended in various salt solutions

Suspending solution			
Solute	M	μ	Specific activity†
K_2SO_4	0.05	0.15	700
KH_2PO_4/K_2HPO_4	*	0.15	797
KCl	0.15	0.15	0
KBr	0.15	0.15	0
KI	0.15	0.15	0

*0.043 M KH_2PO_4, 0.026 M K_2HPO_4.
†Of isocitric dehydrogenase. For test system used, see Table I.

prepared from phosphate washed cells was quite as active as one from sulphate-suspended cells even when the extract was tested in a system containing no

added sulphate. Thus sulphate would appear to have no capacity to activate the system specifically. When other portions of cells from the same growth medium were washed and suspended in halide salt solutions, it is evident that the extracts obtained had no isocitric dehydrogenase activity. Neither of the three halide salts when tested at levels which would be introduced by the extract into the test system showed any capacity to inhibit the system when an extract containing an active enzyme was present.

Although the proper choice of a salt for use as the solute in solutions used to wash and suspend the cells for sonic treatment was essential to obtain isocitric dehydrogenase activity in the resulting cell-free extract, the ionic strength of the solution was found also to have a marked effect upon the activity of the enzyme released. It is clear from Fig. 1 that when a K_2SO_4 solution was

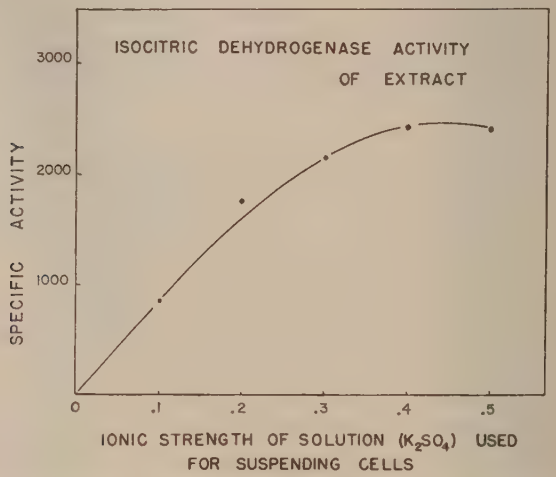

FIG. 1. Effect of ionic strength of medium used for suspending cells during sonic treatment on the activity of isocitric dehydrogenase in the resulting cell extract. Cuvette contents: *dl*-isocitrate (Tris salt), 20 μmoles; TPN, 1 μmole; $MgCl_2$, 50 μmoles; Tris buffer, pH 8. Total Tris concentration, 0.343 *M*. Ionic strength of cuvette contents, ca. 0.38. Cell extract, $\simeq$ 0.25 mg protein/ml. Total vol., 3.0 ml. Temp., 25°.

used to wash and suspend the cells, the specific activity of the extract prepared was nearly proportional to the ionic strength of the suspending solution. Maximum activity was obtained at an ionic strength of about 0.4. This effect of ionic strength during or prior to sonic treatment is independent of the ionic strength requirements for enzyme activation. The extracts prepared from cells suspended at each of the ionic strengths shown as points on the curve in Fig. 1 were in each case assayed for activity under conditions of the same ionic strength, which in this experiment meant adjusting the cuvette contents to 0.38 μ with Tris buffer.

Although KCl present as the sole solute in a solution used to suspend the cells did not give rise to an extract with significant isocitric dehydrogenase activity, it was found to be capable of contributing to the ionic strength

equirements for obtaining an extract with an appreciable specific activity hen combined with a small amount of another salt able to give rise to an ctive enzyme (Table III). It is evident that a combination of KCl contributing 0.1 μ with K_2SO_4 contributing 0.05 μ gave rise to an extract with much

TABLE III

Capacity of a halide salt to contribute to the ionic strength requirements for activating isocitric dehydrogenase from a marine bacterium

Suspending solution		Protein conc.*	Specific activity†
Solute	μ		
K_2SO_4	0.05	13.8	540
	0.15	16.0	2260
	0.30	18.5	3020
KCl	0.10	16.5	22
	0.15	20.0	132
K_2SO_4+KCl	0.05+0.1	17.5	1225

*Of the extracts prepared, in mg/ml.
†Of isocitric dehydrogenase. For test system used, see Table I.

nore activity than one prepared from cells suspended in 0.05 μ K_2SO_4 alone, hough with less than in an extract from cells suspended in a K_2SO_4 solution of he same ionic strength as the combination.

Included in Table III are the protein concentrations of the extracts prepared rom cells suspended in the different salt solutions. A recent study has shown hat a certain minimum salt concentration is required to obtain disruption of ells by sonic treatment (10). It is evident, however, that the ionic strength ffects obtained in this study were not related to the extent of cell disruption s measured by the protein concentrations in the various extracts.

The results obtained so far suggested that there were two requirements for btaining an active isocitric dehydrogenase. One was that a halide not be the sole anion in the medium used to suspend the cells, the other that the medium be of appropriate ionic strength. Since the halide effect appeared not to be a positive inhibitory one it seemed possible that it was due to an inability of halide to protect the enzyme during preparation either from physical stresses imposed during sonic treatment, from one or more components of the extract which might be capable of destroying it, or from spontaneous denaturation.

To determine if the halide effect was specific for extracts prepared by sonic disruption of the cells, extracts were also prepared by two other methods, alumina grinding and lysis by versene and lysozyme. Cells from a medium were divided into two portions, one of which was washed and suspended in a K_2SO_4, the other in a KCl solution. Half of each suspension was then used to prepare a cell-free extract by alumina grinding and half was treated sonically to provide a control. In a separate experiment, extracts prepared by cell lysis were also compared with a control obtained by sonic treatment.

In both cases (Table IV) extracts from cells washed and suspended in the halide solution had little or no isocitric dehydrogenase activity while those from cells suspended in a K_2SO_4 solution were very active. Aconitase activity,

measured in the extracts obtained by cell lysis, was again essentially independent of the solutions used to suspend the cells.

TABLE IV

Comparison of enzyme activity in extracts prepared by alumina grinding, lysis by versene and lysozyme, and sonic treatment from cells suspended in solutions of either KCl or K_2SO_4

Method of preparation	Suspending solution	Specific activity†	
		Isocitric dehydrogenase	Aconitase
Alumina grinding	K_2SO_4	2400	—
	KCl	98	—
Sonic	K_2SO_4	1935	—
	KCl	163	—
Lysis*	K_2SO_4	1876	296
	KCl	0	418
Sonic	K_2SO_4	1877	236
	KCl	97	286

*Lysis by versene and lysozyme.
†For test systems used, see Table I.

Since results obtained with extracts prepared by cell lysis were similar to those found when extracts were prepared by less gentle procedures, it seemed unlikely that physical stresses during preparation of the extract could be responsible for the halide effect. A possible clue to the nature of the phenomenon resulted from the observation that upon occasion an extract prepared from cells washed and suspended in a halide solution did have isocitric dehydrogenase activity. Under such circumstances the enzyme was very unstable and lost most of its activity after storage for 24 hours at 4° C. When the rate of loss of activity of such a preparation was compared with that of a 1:10 dilution of the same preparation, it was found that there was very little difference (Table V). Had the loss of activity been due to the presence in the extract of an agent such as a proteolytic enzyme or heavy metal ion capable of catalytically inactivating the isocitric dehydrogenase and against which sulphate ion pro-

TABLE V

Effect of dilution on rate of loss of isocitric dehydrogenase from extracts prepared from cells suspended in either $MgCl_2$ or $MgSO_4$ solutions

Suspending medium*	Dilution of extract†	Storage time (hr)‡		
		0	24	72
		Activity remaining (%)		
$MgCl_2$	None	100	27	1
	1:10	100	13	1
$MgSO_4$	None	100	85	73
	1:10	100	—	83

*$MgCl_2$ solution 0.05 *M* (0.15 μ), $MgSO_4$ 0.0375 *M* (0.15 μ). Extracts prepared by sonic treatment.
†Dilutions made with solutions used to suspend the cells.
‡Extracts stored at 4° C.

ided protection, dilution of the extract should have reduced the rate of loss of ctivity appreciably. As it was, however, the rate of loss of activity was ssentially independent of the concentration of the enzyme and would thus ppear to be following the kinetics of a first order reaction. One must conclude nen that the enzyme is spontaneously denaturing and that this process proceeds ery much more readily in a halide solution.

Another inorganic factor affecting the stability of isocitric dehydrogenase ame to light in the course of this study. It was found that the presence of Mg^{++} in the solution considerably improved the stability of the enzyme on torage under conditions considered otherwise optimum for maintaining the ctivity of the enzyme. This is shown in Table VI where the addition of 0.05 *M*

TABLE VI

Capacity of Mg^{++} to stabilize isocitric dehydrogenase in a cell-free extract of a marine bacterium

Suspending medium*	Storage time (days)						
	0	1	2	5	6	13	20
	Activity remaining (%)						
K_2SO_4	100	72	48	28	13	0	0
$K_2SO_4+MgSO_4$	100	92	72	60	56	16	10
$K_2SO_4+MnSO_4$	100	91	64	27	19	0	0

*Cells from the same medium were washed and suspended in 0.133 *M* K_2SO_4 (0.4 μ) for sonic treatment. The extract was divided into three portions. Two of these were made 0.05 *M* with respect to either $MgSO_4$ or $MnSO_4$. To the third, sufficient additional K_2SO_4 was added to compensate for the increased ionic strength resulting from the additions to the other two portions. Extracts were stored at 4° C and aliquots diluted appropriately with 0.1 *M* K_2SO_4 for testing. Test system used, see Table I.

Mg^{++} to a cell-free extract prepared from cells washed and suspended in a K_2SO_4 solution, considerably reduced the rate of loss of isocitric dehydrogenase ctivity in the preparation. Mn^{++}, which can replace Mg^{++} in activating the nzyme, showed no significant capacity to replace Mg^{++} in stabilizing it.

Discussion

There would appear to be three separate effects of inorganic salts on the stability of the isocitric dehydrogenase in this organism. First, there is the non-specific requirement for a medium of appropriate ionic strength to maintain the activity of the enzyme. Superimposed on this is the more specific need for sulphate or phosphate ions as opposed to halides to prevent loss of activity. Finally, when both of the above requirements are met, the presence of Mg^{++} causes additional stabilization of the enzyme.

The data indicate that loss of activity of isocitric dehydrogenase when a halide is the sole anion in the medium is most probably due to protein denaturation. It is likely that the other ion effects protect against this same phenomenon.

Enzymatic activity in a protein appears to depend in some way on the manner in which the polypeptide chain and its attached amino acid residues are arranged within and on the surface of the molecule, the whole structural pattern being stabilized by a great number of weak secondary intramolecular bonds

(11). The fact that three separate effects of ions on the stability of the isocitric dehydrogenase reported here have been distinguished suggests that there are at least three separate ways in which the intramolecular bond forces in this protein can be modified to bring about protein denaturation.

Specific effects of anions on the stability of proteins have been reported. Denaturation of ovalbumin in urea solutions was inhibited strongly by such multiply charged anions as sulphate and phosphate and to a much lesser extent by halides (12). Denaturation of ovalbumin by heat, on the other hand, was strongly inhibited by *both* Na_2SO_4 and NaCl (13). Differences in the capacity of anions to activate an enzyme have also been reported (14).

It was at first considered possible that the ability of Mg^{++} to stabilize the isocitric dehydrogenase was related to its capacity to activate the enzyme, possibly by becoming bound at and thus protecting a labile group at the active site. This was rendered less likely by the observation that Mn^{++}, which also activates the enzyme, was unable to stabilize it. Furthermore, it has not been established whether the ability of Mg^{++} and Mn^{++} to activate isocitric dehydrogenase in the crude extract is in reality due to the requirement of an oxalosuccinic carboxylase for these ions as is the case with the pig heart enzyme (7). It may be of significance in connection with the Mg^{++} effect that $CaCl_2$ was found to be much more effective than chlorides of monovalent cations in preventing denaturation of the lactic dehydrogenase from a halophilic bacterium (15). Unfortunately, Ca^{++} could not be tested in the system reported here because of the attendant requirement for sulphate or phosphate for stability. That one cannot generalize regarding the capacity of alkaline-earth cations to prevent denaturation, however, is evident from the fact that Ca^{++}, Mg^{++}, and Ba^{++} salts have been observed to accelerate the denaturation of ovalbumin in urea solutions (11).

In one recent study, isocitric dehydrogenase activity could not be demonstrated in extracts of *Acetobacter peroxidans* (16). Based on this observation and failure to demonstrate the oxidation of citrate and α-ketoglutaric acid, it was concluded, with reservations, that terminal acetate oxidation in this organism takes place by a dicarboxylic rather than by a tricarboxylic acid cycle. In another study with the same organism, evidence for isocitric dehydrogenase activity was obtained (17). In the investigation where isocitric dehydrogenase could not be detected, cells of the organism had been washed and suspended in water prior to sonic treatment, while where it had been found, the cells had been washed and suspended in phosphate buffer. In view of the present findings, it seems not unlikely that this difference in procedure could have been responsible for the discrepancy in the results obtained.

It would appear that there is no way of predicting the capacity of electrolytes to affect the activity or stability of an enzyme. Awareness of their possible importance in permitting the detection of an enzyme is desirable, however, when conclusions regarding the pathway of metabolism in an organism is to be based on failure to detect a key enzyme.

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Proximate Analysis of Marine Standing Crops

In most circumstances the bulk of the biomass in a sample of sea-water consists of microscopic particles that pass the coarser plankton nets and its analysis, after the manner of the proximate analyses made in agricultural chemistry, is becoming increasingly important in assessing marine food potential. At present there exist no really satisfactory methods for doing this and the only commonly used procedures are pigment extraction methods[1] which gives some measure of living plant material, and the tedious and time-consuming processes of direct microscopy. Methods such as Krey's weighing techniques[2] are difficult to apply in the field and give no measure of the food potential of the solids being weighed.

The main problem is one of the quantitative collection of particles as small as 1μ in diameter from many litres of water. This is solved conveniently by filtration through a membrane 'sieve' filter, such as the 'Millipore' *HA* or *AA* filter disks. The difficulty is then one of removing the collected crop from the membrane surface without contaminating it with traces of the cellulose base filter. With natural crops or cultures of the larger diatoms the cells can often be removed quite readily by washing them off the filter. However, the ultra-plankton and much detrital matter stick hard to the membrane surface. We have found that if 1–2 ml. of a 1 per cent suspension of fine magnesium carbonate are diluted with about 50 ml. of water and then drawn on to the membrane by sucking it dry, the resulting 'gravel bed' does not impair the filtration speed of a 'Millipore' filter and enables filtered particles, whatever their nature, to be removed quite readily. There is little or no adsorption of soluble organic material. However, blanks should be run, using fresh membranes, magnesium carbonate and a little distilled water, as traces of organic matter may still be removed.

Samples of sea-water, pumped from the euphotic zone or obtained from greater depths by bottle casts, are best collected in a large polyethylene vat and subsampled into polyethylene bottles for filtration. If the water (in amounts not exceeding a few litres) is first poured through a clean nylon net of about 0·2-mm. mesh size the larger zooplankters are removed with only a small percentage loss of phytoplankton cells.

A volume of sea-water suitable for analysis can be passed through a 47-mm. diameter *AA* 'Millipore' disk, coated with magnesium carbonate, in less than 20–30 min. (0·5–2 l. in coastal areas or up to 5–10 l. in the open ocean). The membrane is washed completely clean by placing it on the side of a 30-ml. beaker and washing off the precipitate by short hard bursts of 3·5 per cent saline. A proximate micro-analysis of the filtered material can then be made, after centrifugation, using rapid and sensitive spectrophotometric methods on aliquots of the centrifuged solids.

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Table 1

Sample	Oxidizable Carbon (mgm./m.³)	Carbohydrate (as glucose) (mgm./m.³)	Protein (as egg albumin) (mgm./m.³)	Lipid (as stearic acid) (mgm./m.³)	Chlorophyll *a* (mgm./m.³)	Plant Carotenoids (m. *SPPU*/m.³)
49° 13′ N. 123° 57′ W. Departure Bay (Nanaimo), April 21, 1959.	475	550	398	43	12	4·5
Culture of ocean phytoplankton (mixed coccolithophores and small diatoms).	730	455	325	91	11·3	3·7
50° N. 145° W. 0–50 m. composite sample August 4, 1959.	130	72	150	22	0·27	0·08

We have analysed for carbohydrate, using the anthrone reagent[3] and for fats using the pinacyanole reagent of Mukerjee[4]. Protein is conveniently assayed using the Folin–Coicalteu reagent with or without the addition of the biuret copper reagent[5]. Pigments are best determined on separate samples by the Richards method[1]. We have devised a convenient method of measuring oxidizable carbon using a separately filtered sample. Instead of centrifuging the mixture of cells and magnesium carbonate that have been washed from the 'Millipore' membrane, the suspension is refiltered at once through a 1-in. diameter *UF* porosity glass disk held in a 'Millipore' 1-in. filter holder. This disk is rinsed once with water and transferred to a small glass beaker. Heating on a boiling water bath with a little concentrated phosphoric acid is sufficient to remove any chloride interference and the residue is then treated with a chromic acid—sulphuric acid oxidant[6], the reduced chromate being estimated spectrophotometrically. Full details of these methods are to be published.

Table 1 shows typical analyses taken from north-east Pacific coastal and ocean waters. At the coast the solids consisted largely of phytoplankton cells. In the open ocean the level of organic matter was not very different, but the plant cells contributed only a small fraction of the whole, as witnessed by low pigment to carbon ratios. This particulate matter, which microscopical examination indicated was largely detrital in nature, persisted to many hundred metres. A significant amount of chitin may have been present but this is not estimated by the use of anthrone. The fat contents of all samples are lower than would be expected from data in the literature[7–9]; but it must be remembered that the present technique measures only soap-forming lipid material and not the 'ether extractable' matter normally classed as fats, which might well be expected to be much greater in both living cells and detritus.

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DEFINITION OF HADDOCK STOCKS OF THE NORTHWESTERN ATLANTIC

BY JOHN R. CLARK AND VADIM D. VLADYKOV

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ABSTRACT

Differences in average vertebral number, related to temperature on the spawning grounds, have been used to separate northwestern Atlantic haddock into five major stocks: Newfoundland, eastern Nova Scotian, central Nova Scotian, western Nova Scotian, and New England.

FINITION OF HADDOCK STOCKS OF THE NORTHWESTERN ATLANTIC

By JOHN R. CLARK, *Fishery Research Biologist*, and VADIM D. VLADYKOV, *Fishery Biologist*[1]

ıddock are distributed over the continental
es of the North Atlantic Ocean and in no
waters. They are not distributed continu-
over this vast range, but reside in certain
s delimited by topographic and hydrographic
daries. Nor are they evenly distributed
in any area. Haddock generally prefer
hs of less than 110 fathoms. They are pres-
considered to belong to a single species,
nogrammus aeglefinus (L.).

the northwestern Atlantic, haddock appear
ertain fishing grounds only during the winter
pring for spawning. Some grounds support
eries for haddock only during the summer and
umn while others are fished throughout the
r. The character of populations sustaining
-round fisheries often varies from season to
on, with older fish appearing for spawning
younger fish replacing them during the re-
nder of the year.

.ge composition, growth rate, and other char-
ristics of haddock population units differ
m place to place, causing us to believe that the
cies consists of many more-or-less separate
ts. This idea is strengthened by tagging ex-
iments in the western Atlantic which have
vn that haddock, although migrating over
er definite routes, infrequently cross the deep-
r channels separating the New England,
va Scotian, and Newfoundland Banks (fig. 1).
the basis of such evidence, Needler (1930)
gested a subdivision of northwestern Atlantic
ddock into three major units: the New Eng-
d, Nova Scotian, and Newfoundland stocks.
rther research has, in general, substantiated
s division (Schroeder 1942; Schuck and Arnold,
1; Vladykov 1935). Each of the three regions
encompasses a number of distinct grounds which support important fisheries at various times of the year.

Properties of the environment, notably temperature, differ from one fishing ground to another. These properties may be expected to significantly modify the meristic characters of haddock as they do of other species. Notwithstanding the effect of genetic influences, the differences in these characters should permit identification of broods originating in different localities. It is the purpose of this paper to demonstrate that one meristic character, the number of vertebrae, can be used to identify haddock stocks.

COLLECTION OF DATA

Our collection of vertebral counts was begun in 1932, and a preliminary study was published (Vladykov 1935) which demonstrated recognizable differences for the haddock of various western Atlantic fishing grounds. Since then the Fisheries Research Board of Canada and the U.S. Fish and Wildlife Service have added many thousands of counts to the collection, representing altogether the haddock of 16 major fishing grounds from New England to Newfoundland. The samples have come from the catches of commercial fishermen and research vessels using otter trawl and hook-and-line gear.

The entire collection of data is detailed in table A–1 of the Appendix, p. 294. In counting the vertebrae, all of them from the first vertebra, found immediately behind the basi-occipital bone, to the penultimate one, which has a normal centrum and a hypural plate, are included. The last vertebra with its modified centrum and terminal hypural plate is not included in our counts. Fish having vertebral abnormalities, such as fused centra, amounted to slightly more than 1 percent of the total number of specimens and were not used.

...TE.—John R. Clark, North Atlantic Fishery Investigations, Bureau of Commercial Fisheries; Vadim D. Vladykov, Department of Biology, University of Ottawa, Ottawa, Ontario.
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...e paper is based partly on data collected by the Fisheries Research Board ...nada, and worked on by Dr. Vladykov while in the employ of the Board.

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FIGURE 1.—The submarine physiography of the Continental Shelf from Labrador to Cape Cod. (Adapted f Hachey, Hermann, and Bailey, 1954.)

GEOGRAPHICAL VARIATION IN VERTEBRAL NUMBERS

In studies of many species, the number of vertebrae has been shown to differ significantly among geographically separated units of the population. The number of vertebrae typically increases with latitude, suggesting an inverse relation with water temperature.[1]

To investigate the possibility of such a relation between number of vertebrae and water temperature for haddock, we grouped our samples by fishing ground and calculated the average number of vertebrae for each (appendix table A–2). A difference of nearly 1.3 vertebrae is shown between the highest and lowest averages. Analysis-of-variance treatment of the data (see append showed these differences between the ground be highly significant ($F = 182$).

As shown in figure 2, the number of verte tends to increase from Georges Bank north erly along the Scotian Shelf. The average St. Pierre Bank, however, is lower than even one for Georges Bank.

Another presentation of these data is give figure 3 where we have plotted, after the met of Hubbs and Perlmutter (1942), the main st tical values for the data from each fishing grou total range of items, standard deviation, m and two standard errors either side of the m Here we see that numbers of vertebrae incr regularly from Georges Bank to Emerald Ba hold relatively constant from Emerald Ban the Gulf of St. Lawrence, then drop sharpl St. Pierre Bank. The significance of these di ences is shown by comparison of the stand errors of the various distributions.

[1] See, for example, Schmidt 1930, Thompson 1943, Hansen 1949, for cod; Bertelsen 1942, for coalfish; Tåning 1929, Devold 1942, for plaice; Runnström 1941, Tester 1949, McHugh 1954, for herring; Hansen 1943, Templeman 1948, for capelin; Clark 1947, for sardine; Hubbs and Perlmutter, 1942, McHugh 1951, for anchovy; Vladykov 1934, for summary of earlier information.

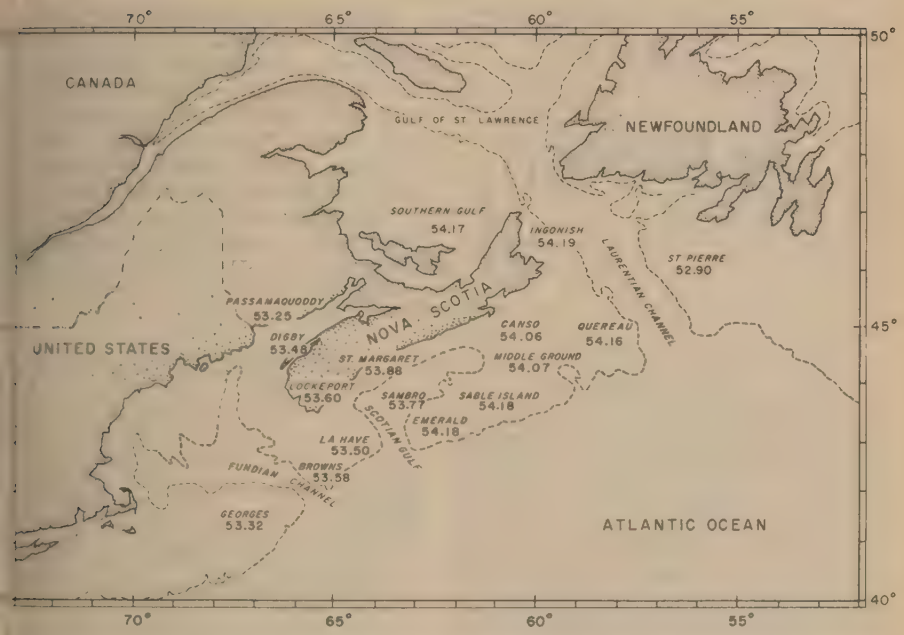


Fig. 2. Haddock fishing grounds of the northwestern Atlantic and the mean number of vertebrae of haddock from each.

VERTEBRAL NUMBERS AND WATER TEMPERATURE

Since water temperatures decrease from Georges Bank northeasterly along the coast, we see that these conform in general to the typical negative correlation of meristic characters and temperatures. To discover how close this correspondence is we must examine the temperature regime on a spawning ground during the time when the number of vertebrae is actually established.

It is probable that vertebral numbers are established within the first week or so following fertilization of the eggs. We do know for certain that vertebrae are formed by the time the fish are 10 mm. in length, which requires 5 or 6 weeks. Although haddock spawn on or near the bottom, their eggs are buoyant and rise toward the surface following fertilization.[2] Since the eggs tend to be concentrated near the surface, the temperatures of the surface layer are most appropriate. We have, therefore, gathered such information as is available on surface water temperatures during the spawning period of northwestern Atlantic haddock to compare with their average numbers of vertebrae.

Haddock spawn in greatest numbers in mid-March in the New England area, in late March and early April in the Nova Scotian area, and in late May and June in the Newfoundland area.[3] Unpublished surface temperature records representing all available observations during these spawning times were obtained from the Woods Hole Oceanographic Institution. These data are plotted as isothermic lines in figure 4. The temperature for each fishing ground has been estimated from the chart and listed along with the

[2] Bigelow and Welsh (1925) for a discussion of the distribution and development of the early stages of northwestern Atlantic haddock.

[3] Needler 1930; Vladykov and Homans, 1935; Bigelow and Schroeder, 1953; Thompson 1939; and unpublished records of the U.S. Fish and Wildlife Service

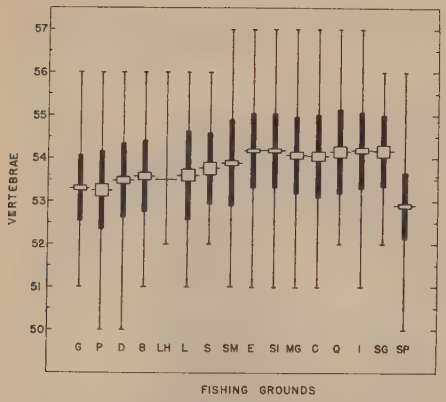


FIGURE 3.—Numbers of vertebrae of haddock from fishing grounds of the northwestern Atlantic. The vertical line represents the range; the solid bar, one standard deviation on each side of the mean; the hollow rectangle, two standard errors on each side of the mean; and the crossbar, the mean. The fishing grounds shown are Georges Bank (G); Passamaquoddy Bay (P); Digby (D); Browns Bank (B); La Have Bank (LH); Lockeport (L); Sambro Bank (S); St. Margaret's Bay (SM); Emerald Bank (E); Sable Island Bank (SI); Middle Ground (MG); Canso Bank (C); Quereau Bank (Q); Ingonish (I); Southern Gulf of St. Lawrence (SG); St. Pierre Bank (SP).

appropriate spawning time in table 1. A negative relation between temperature and vertebral number is demonstrated by the data. The apparent anomaly for St. Pierre Bank is now easily explained by the high water temperature obtaining there during the spawning time (May–June).

TABLE 1.—*Mean number of vertebrae and average surface water temperature during spawning time for haddock of the northwestern Atlantic*

Fishing ground	Temperature (° F.)	Mean number of vertebrae
St. Pierre Bank	43.5	52.90
Georges Bank	38.0	53.32
Digby	37.0	53.48
Passamaquoddy Bay	36.5	53.25
Browns Bank	36.5	53.58
La Have Bank	36.0	53.50
Emerald Bank	35.0	54.18
Lockeport	35.5	53.60
Sambro Bank	35.0	53.77
Sable Island Bank	34.5	54.18
St. Margaret's Bay	34.0	53.88
Middle Ground	34.0	54.07
Canso Bank	33.5	54.06
Quereau Bank	33.0	54.16
Ingonish	32.0	54.19
Southern Gulf of St. Lawrence	32.0	54.17

Some data are available for the northeast Atlantic for comparison with northwestern lantic data. Tåning (1935) listed average n bers of vertebrae that show little relation to l tude in the North Sea, Ireland, Iceland, Far and Norway. Nor could Saetersdal (1952) a relation in his data between latitude and ve bral numbers along the Norwegian coast. H ever, upon treating spawning-time temperat and vertebral averages for these northeastern lantic data in the manner described previously our data,[4] we find evidences of a relation betw the two (table 2 and fig. 5).

TABLE 2.—*Mean number of vertebrae and average temperature during spawning time for haddock o northeastern Atlantic*

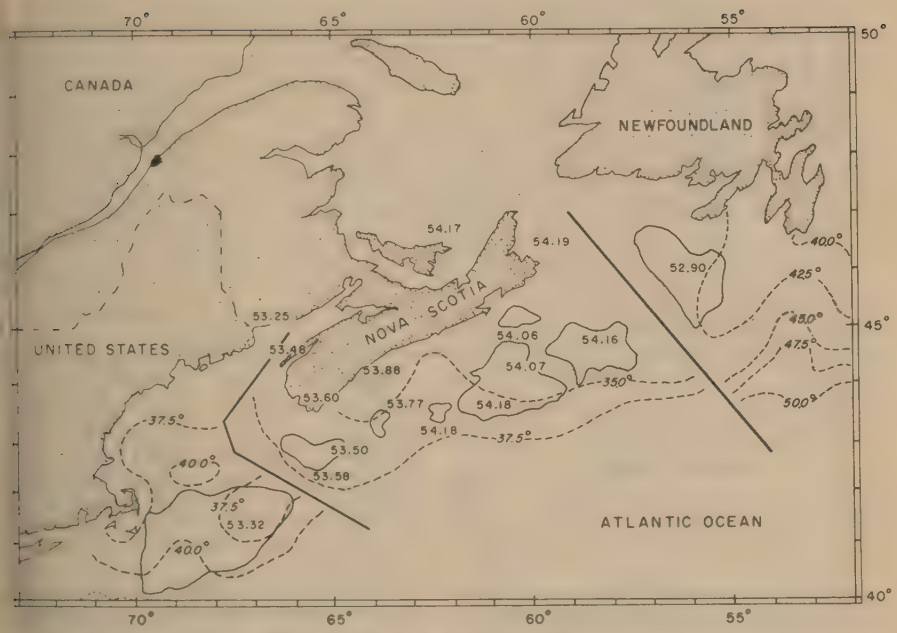
Area [1]	Temperature (° F.)	Me numb verte
Ireland	47.5	
Faroe Islands	45.0	
Iceland	44.0	
North Sea	43.0	
Norway	41.0	
Norway [2]	41.0	

[1] From Tåning (1935).
[2] A single average was calculated where ranges were given or more th mean was presented in the original publication.
[3] From Saetersdal (1952).

The average vertebral numbers for hadd from both sides of the Atlantic have been plo against surface water temperatures at spawn time and a curve has been fitted to them (fig. The data thus assembled include the entire kn range of spawning-time temperatures of hadd

A similar relation between average numbe vertebrae and temperatures at spawning tim shown for both sides of the Atlantic. This, gether with the overlapping range in verte numbers of the Newfoundland and northeas Atlantic samples, suggests that a single verte number-temperature relationship applies to species throughout its range. Temperature w then appear to be an important factor in variation in number of vertebrae of haddock.

[4] Data on spawning times of northeastern Atlantic ha have come from Schmidt 1909, Wiborg 1950, Thompson and Raitt 1948. These data indicate that haddock spaw greatest numbers off Norway and in the North Sea in March, off Iceland and Ireland in mid-April, and off the Islands in early May. Temperature data are from C Permanent International pour l'Exploration de la Mer, (and U.S. Hydrographic office (1944).



URE 4.—Mean numbers of vertebrae of haddock from fishing grounds of the northwestern Atlantic and surface water temperatures (°F.) during spawning time.

VERTEBRAL NUMBERS AND YEARLY ARIATIONS IN WATER TEMPERATURE

The evidence presented suggests that annual ations in water temperature in any particular ality would cause haddock originating in t locality to have varying numbers of verte- e. Temperature at time of spawning and rage number of vertebrae for individual year sses have been successfully correlated for ring by McHugh (1942), Rounsefell and Dahl- n (1932), and Tester (1937, 1938, and 1949). Data are not available with which to assign es to most of our specimens, and the year sses must for the most part be treated together. me material, however, was available to evalu- e differences among certain year classes on orges and Browns Banks. This material was lected during two trips of the *Albatross III* in spring and autumn of 1950. We obtained 369 ecimens from Browns Bank and 974 specimens from Georges Bank which were taken over widely separated areas of each bank and so adequately represented the population present at the time. The ages of the fish were determined from their scales. The vertebral numbers are given in table 3 for each year class. Since the reliability of age determination from scales is uncertain for haddock over 8 years of age, we have grouped the older fish into a single category (9+) and have not assigned them to year classes.

Analysis-of-variance treatment of the data showed the difference between average vertebral numbers from the two banks to be highly significant ($F=10.51$).

The difference between year classes is highly significant only for Georges Bank ($F=4.63$). Although the Browns Bank year classes are not shown by analysis of variance to differ significantly ($F=1.82$), we have used them since the magnitude of the differences was even greater than for Georges Bank.

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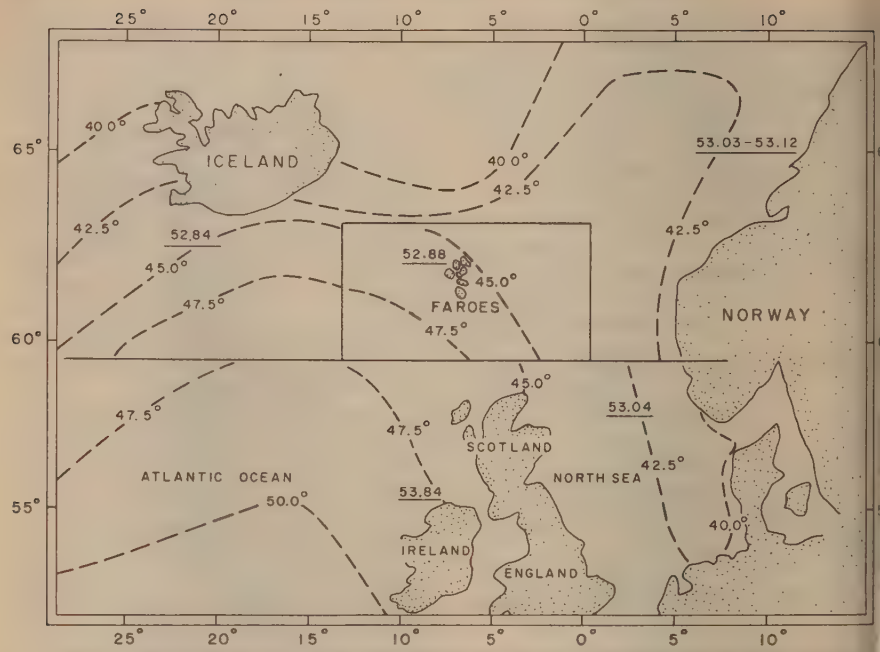


FIGURE 5.—Mean numbers of vertebrae of haddock from the northeastern Atlantic and temperatures (°F.) dur spawning time.

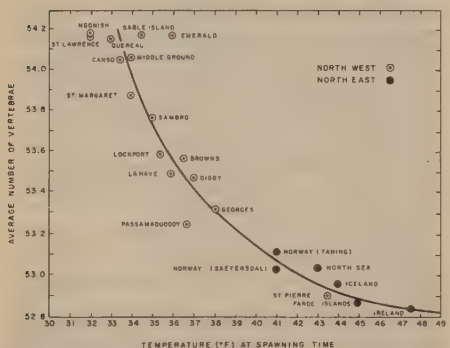


FIGURE 6.—The relation between water temperature during spawning time and numbers of vertebrae of haddock.

No series of temperature observations is available directly from Browns and Georges Banks for the period 1942–49 to use in correlating with vertebral averages of the year classes. A continu series of records is available (U.S. Coast Geodetic Survey, 1947), however, for surf water temperatures at Eastport, Maine, in Pas maquoddy Bay, near the entrance to the Bay Fundy. Hachey and McLellan (1948) have d onstrated that temperatures for Passamaquo Bay give a reliable measure of annual temp ture variation along the northwestern Atla coast.

To utilize these data from Eastport, Maine found it necessary to relate them to the temp tures on the two banks for individual years. average surface water temperature over Bro Bank during the time of haddock spawnin shown in table 1 to be about 36.5° F., and average water temperatures at Eastport for same period of the year are shown in table be the same. The average surface tempera during the spawning time on Georges Bank, l

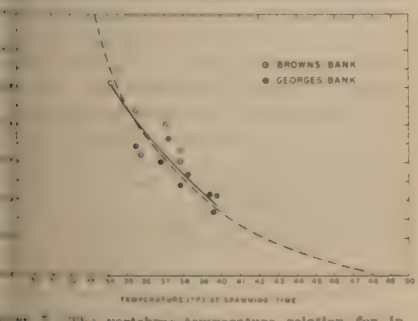
TABLE 3.—*Vertebral-number frequencies for each age of haddock from Georges and Browns Banks*

Age (years)	Year class	Georges Bank								Browns Bank							
		Number of fish	Occurrence of vertebrae numbering						Mean	Number of fish	Occurrence of vertebrae numbering						Mean
			50	51	52	53	54	55	56		50	51	52	53	54	55	56	
	1949	32			5	17	8	2		53 22	1				1			
	1948	381		1	24	153	173	30		53 54	41			3	18	19	1	53 44
	1947	76	1		10	42	22	1		53 14	76		1	6	31	32	6	53 47
	1946	137	1	1	19	59	44	13		53 34	100		1	6	35	48	9	1	53 61
	1945	194		5	25	95	60	9		53 22	60		1	5	29	19	6		53 40
	1944	82		2	6	40	26	7	1	53 40	50			4	15	24	7		53 68
	1943	39			3	19	13	3	1	53 49	30			1	11	11	6	1	53 83
	1942	15			1	10	3	1		53 27	4				3				
		18			2	5	10	1			7		1		3	1	2		
		974	2	9	95	440	359	67	2	53 39	369		4	25	145	156	37	2	53 55

r, approximates 38° F. while temperatures at
'port for the same period average 35.6° F.
peratures on Browns Bank can therefore be
mated directly from readings at Eastport,
estimates for Georges Bank require an adjust-
t of about 2.5° F.

An average of the April readings for Eastport
, therefore, used to approximate spawning
temperatures on Browns Bank. The March–
il average for Eastport was increased by 2.5°
to estimate spawning time temperatures for
rges Bank.

The temperature estimates thus obtained are
ed for the years 1942 to 1949 in table 4, along
the average number of vertebrae for the
ddock spawned in those years. The 1942 and
year classes for Browns Bank are repre-
ted by too few specimens to give meaningful
lts and have not been included. These data
cate a close correlation between water tem-
ature and number of vertebrae of the year



E 7. The vertebrae–temperature relation for in-
vidual year classes from Georges and Browns Banks.
Dashed line from fig. 6.)

classes. How this compares with the previous relationship (fig. 6) can be seen in figure 7, where we have imposed the temperature-vertebrae data for our year classes from Browns and Georges Banks over the curve from figure 6. The curve fitted to the data from the Georges and Browns year classes deviates very little from the curve representing all the fishing grounds.

TABLE 4.—*Estimated surface water temperature at spawning time and mean number of vertebrae for certain year classes of haddock from Georges and Browns Banks*

Year class	Georges Bank			Browns Bank	
	March–April mean temperature at Eastport (°F.)	Adjusted mean temperature (°F.)[1]	Average number of vertebrae	April mean temperature at Eastport (°F.)	Average number of vertebrae
1949	37.4	39.9	53.22		
1947	37.2	39.7	53.14	38.0	53.47
1945	37.0	39.5	53.22	37.9	53.40
1946	35.8	38.3	53.34	37.2	53.61
1942	35.4	37.9	53.27		
1948	34.8	37.3	53.54	35.8	53.44
1944	34.4	36.9	53.40	35.6	53.68
1943	33.0	35.5	53.49	34.3	53.83
Mean	35.6			35.5	

[1] See text for explanation.

IDENTIFICATION OF POPULATION UNITS

It appears from the consistency of the vertebral number-temperature relation that we should be able to predict, within limits, the average number of vertebrae of haddock from any area if we know the temperature at spawning time. The deviations from the curve in figure 6 may represent fish not spawned on the grounds where they were captured. In like manner, the points falling near the line may represent stocks which were spawned on the grounds where captured or on

grounds having similar temperatures at spawning. Some interesting points may be brought forth by considering the data for each fishing ground with these suggestions as an hypothesis:

St. Pierre Bank.—The data fit closely to the curve, indicating negligible movement of other haddock to this area.

Georges Bank.—Following the same reasoning as for St. Pierre, Georges Bank haddock appear to reside in the area of their origin with no significant immigration.

Passamaquoddy Bay.—Haddock are known to be summer visitants only to this area, with spawning taking place elsewhere. Their average vertebral number indicates that they are probably hatched in waters having temperatures higher than 38° F. As water of this temperature is usually found in the western Atlantic only in the vicinity of Georges Bank during the spawning time of haddock (see fig. 4), we may assume that the Passamaquoddy haddock were spawned in the George Bank area, perhaps slightly north of the western part of the bank proper. Needler's study (1930) of age and length composition and growth rates showed Passamaquoddy and Georges Bank haddock to be closely related. Needler's tag-return data, as well as recent unpublished United States and Canadian tag-return data, further show that Passamaquoddy haddock generally move south in the winter.

Browns Bank, Digby, Lockeport, LaHave and Sambro Banks, St. Margarets Bay, Canso Bank, Middle Ground, and Quereau Bank.—Average vertebral numbers fit well to the curve for haddock of these grounds indicating that they reside as adults near their original birthplace. In a study of greatly increased haddock catches in the western Nova Scotian area in 1938, McKenzie and Homans (1939) found subnormal numbers of vertebrae, indicating recruitment from New England stocks. Thus, occasional movement of New England haddock via the shoal water of the Bay of Fundy to Nova Scotian waters has been shown to be a possibility. But this is the only instance of such a population shift that has been brought to our attention.

Emerald and Sable Island Banks, Ingonish, and Southern Gulf of St. Lawrence.—Averages do not fit the curve well for haddock of these haddock grounds indicating that they have been hatched in an area where spawning temperatures would average about 33.5° F. rather than in areas where they were caught. Waters of t temperature may be found over certain of eastern Nova Scotian Banks. Our method wo indicate therefore that these four groups of h dock have a common birthplace on the eas part of the Scotian Shelf where temperatu from 33° to 34° F. are to be expected during spawning period. This conclusion is parti substantiated by the tag studies of Needler (19 who discovered that haddock appearing on eastern Nova Scotian Banks during the Sp spawning period migrated in summer and fal the Ingonish area and into the Gulf of Lawrence.

We now have sufficient evidence to reexan the problem of grouping northwestern Atla haddock into major subdivisions. The discuss of individual fishing grounds suggests that n than three independent units (New Engl stock, Nova Scotian stock, and Newfoundl stock) may be recognized. If we group grounds on the basis of vertebral average redefinition of the major units is suggested.

The choice of words to be used in defi population units is always difficult, and we c not to concern ourselves too greatly with semantics of such terminology. We shall fo the nomenclature suggested by Marr (1957), describe our subdivisions as "stocks."

The five major units of the northwestern lantic haddock population which may at pre be identified are as follows:

1. The Newfoundland stock, which can clearly separated from all others on the basi the very low number of vertebrae of had from St. Pierre Bank and vicinity. As sam from Grand Bank proper are not available. status of these fish must still remain in quest

2. The eastern Nova Scotian stock, which tends east to the Laurentian Channel and we the Scotian Gulf. An inshore group on C Bank and Middle Ground may be distingui from the offshore group on Emerald, S Island, and Quereau Banks, and in the Gul St. Lawrence. The differences appear too sli however, to justify further subdivision at pre

3. The central Nova Scotian stock, whic represented in this study by the St. Marga Bay and Sambro Bank groups, and resides in vicinity of the Scotian Gulf.

The western Nova Scotian stock, which re- in the area bounded on the east by the n Gulf and on the west by the Fundian nel. This stock includes fish from Locke- nd Digby, LaHave, and Browns Banks. The New England stock, which includes fish iting grounds west of the Fundian Channel long the New England coast and is repre- i in this study by the Georges Bank and maquoddy Bay samples.

DISCUSSION

r data do not permit us to go beyond the of suggesting the general arrangement of . The actual degree of mixing between units of the population as we have suggested t be estimated from the average numbers of brae and such considerations are not within ope of this analysis. A gradual mixing of antial proportions of adjoining stocks of planktonic stages, however, should cause the bral numbers of the older ages to differ less those of the younger ages. An increase in bral averages would be shown for the more erly stock, and the reverse would be shown he more northerly stock. This was not true our year-class samples from Georges or ns Banks, however, and the relation between erature and vertebrae remained constant gh the range of ages.

ether refinement of our method will permit precise identification of components of the r stocks must await the results of further rch that is now in progress. Recent success ging haddock offshore should provide direct nce on the question of intermixture of units. rect confirmation of our vertebral number- erature relationship must await experimen- hatching and rearing. Haddock eggs are n to develop in water as cold as 30° F. and rm as 60° F. (personal communication, John Colton, Jr.). We could thus experimen- extend the relation past the range of our nt observations which are based on natural ning conditions.

SUMMARY

ertebrae of haddock from 16 fishing grounds he northwestern Atlantic were counted to de- termine whether such counts could be used to identify population units.

Comparison of average numbers of vertebrae from the different areas showed significant differences, which could be related to surface water temperatures on the grounds during the spawning time. The relationship is consistent with that developed for northeastern Atlantic haddock from published European data.

Age data which were available for the samples from Georges and Browns Banks demonstrated that the differences in average vertebral numbers among the individual year classes on each bank could be attributed to differences in temperatures in the spawning period in different years.

Consideration of average vertebral numbers of the various population units suggests the following as major haddock stocks of the northwestern Atlantic: Newfoundland, eastern Nova Scotian, central Nova Scotian, western Nova Scotian, and New England.

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APPENDIX

The entire collection of vertebral counts used in this study is given by fishing ground and date in table A–1. A summary of the frequency distributions of the vertebral counts for the various fishing grounds and related statistical values is presented in table A–2.

Analysis of variance was used to test differences among grounds and among samples of the various grounds. The original total of 9,985 vertebral counts composed of 108 samples from 16 grounds were used in the analysis. The results are as follows:

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Total	9,984	9822.30		
Between grounds	15	2112.30	140.8200	182.08
Between samples	9,969	7710.00	0.7734	
Between samples	107	145.02	1.3553	1.77
Within samples	9,862	7564.98	0.7671	

The F value of 182.08 shows that differences among the grounds are highly significant (probability of occurrence by chance less than 0.01). Individual tests, comparing differences between pairs of grounds, can be made with the analysis of variance, but to make all possible comparisons between the 16 grounds is not practicable. Individual comparisons were made between units of different major stocks which are adjacent to each other but separated by the channels because these are of particular importance.

Highly significant differences were found between Georges and Browns Banks ($F=15.52$), between Sambro and Emerald Banks ($F=26.80$), and between St. Pierre and Quereau ($F=486.87$). These results indicate that population units representing different stocks but adjacent to each other and bordering on the channels are clearly distinct from each other.

The highly significant F value of 1.77 for the between-samples treatment indicates that the variation among samples for particular grounds is real, and the samples have been drawn from different populations.

Such real differences as do exist may be explained partially by seasonal and annual changes in year-class composition on the grounds. The only data available to test this hypothesis are those for which age data are available; namely, the 1950 collection for Georges and Browns Banks. These data are shown in text table 3. Analysis-of-variance treatments showed that the differences between individual year classes on Georges Bank were highly significant ($F=4.63$). The year classes on Browns Bank did not differ significantly ($F=1.82$), although the magnitude of difference was even greater for Browns than for Georges year classes. We would expect such differences to occur on other grounds as well.

Year-class composition on any ground will change with time. The mean vertebral number can also be expected to change since it depends on the proportion of various year classes represented. This is the most probable explanation for the significant difference between samples.

TABLE A–1.—*Vertebral-number frequencies of haddock, by fishing ground and date of collection*

Fishing ground and date of collection	Number of fish with vertebrae totaling—								To
	50	51	52	53	54	55	56	57	
Georges Bank:									
November 1932		1	19	55	32	2			
June 1935			8	36	18	1			
Do.			1	10	10	1			
Do.			9	27	14	1			
Do			3	20	15	5			
February 1939		4	50	172	110	10	1		
March 1949				4	2				
April 1950		2	23	113	105	18	1		
August 1950	2	7	72	327	254	49	1		
Total	2	14	185	764	560	87	3		
Passamaquoddy Bay									
July–August 1933			6	14	13	2			
September 1933	1	2	19	55	39	5	2		
Total	1	2	25	69	52	7	2		
Digby									
December 1933			7	14	24	5			
October 1934		2	13	46	50	20			
December 1935	1	1	13	65	37	6	1		
May 1936			9	44	48	14	1		
Total	1	3	42	169	159	45	2		
Browns Bank									
April 1935		1	17	72	64	18	1		
July 1936			2	12	6				
March 1939		1	16	67	54	10	1		
March 1949			2	2	3	1			
May 1950		3	5	69	64	22	2		
August 1950		1	20	76	92	15			
Total		6	62	298	283	66	4		
La Have									
June 1936			4	5	9	1	1		
Lockeport									
December 1930		3	9	34	25	19	5		
November 1934		1	10	39	40	13	1		
Total		4	19	73	65	32	6		
Sambro									
July 1933			2	10	10	3			
October 1934			5	30	50	17	2		
Total			7	40	60	20	2		
Halifax and St. Margaret's Bay:									
July–August 1932			2	10	7	10	3		
July–October 1932		1	4	48	64	45	9		
June–September 1933			3	17	24	11	3		
July 1933			5	23	26	18	3		
August 1933		1	8	29	34	10	1		
November 1934		1	5	16	18	8	3		
Do		1	5	28	19	3	1		
Do		1	5	11	18	12	2		
Do			1	18	20	9	1		
December 1934			9	27	32	17	6		
May 1935		2	8	39	65	37	6		
Do			4	30	60	21	5	1	
Do			7	30	38	30	2	2	
Do			6	19	43	29	4	1	
Total		7	72	345	468	260	49	4	
Emerald Bank									
September 1934			1	10	7	5	1		
January 1935			2	7	23	20	4		
Do			2	17	64	36	4		
February 1935			3	12	47	30	5		
Do			5	24	65	47	7		
Do			3	16	46	38	3		
Do			3	18	39	23	5		
March 1935			3	16	43	28	10		
Do			3	12	45	31	5		
Do			2	14	50	32	4		
April 1935			3	28	73	60	10		
Do			1	10	40	36	6		
May 1935		2	1	8	36	25	2		
Do			1	15	51	29	4		
Do				19	40	34	6		
June 1935			5	20	46	22	2		
March 1936		1	4	20	39	32	2		
Do			5	31	63	39	4		
April 1936			5	21	38	21	4		
Total		3	52	318	855	588	88		
Sable Island:									
August 1933			1	12	44	42	2		
September 1934			3	30	74	64	9		
Do		1	2	32	65	39	8	2	
Do			4	17	37	39	1	1	

TABLE A 1.– *Vertebral-number frequencies of haddock, by fishing ground and date of collection* Continued

ng ground and date of collection	Number of fish with vertebrae totaling—								Total
	50	51	52	53	54	55	56	57	
nd Continued									
er 1934		1	1	35	65	52	8		162
o			2	17	44	26	4		93
o			1	10	32	31	6		80
o			3	19	28	23	6		79
mber 1934			1	10	14	12	2		39
o				6	20	8	1		35
o			1	6	28	22			57
o			6	16	22	21	3		68
o				5	20	12			37
o			2	8	14	11			35
1935		1	1	10	28	21	5		66
		1	2	19	53	15	7	1	108
				23	43	26	4		96
ary 1936			2	17	74	40	4	1	138
tal		4	32	292	715	504	70	5	1,622
Ground									
er 1934			2	17	65	37	1		122
st 1935		4	17	52	106	69	4	1	253
1936			1	12	40	45	1		99
tal		4	20	81	211	151	6	1	474
nk and Petit Degrat									
1934			2	7	10	4	3		26
mber 1934			1	2	8	5			16
Dec 1934		1	5	27	35	23	1	1	93
1935		1	1	11	25	12	3		53
mber 1935		1	2	14	42	31	1	1	92
1936			3	5	29	15	4	1	57
otal		3	14	66	149	90	12	3	337
Bank									
ember 1934			1	10	14	8	2		35
vember 1934			4	18	45	26	6		99
Do			6	14	40	30	8	2	100
Do			6	29	65	37	5		142
otal			17	71	164	101	21	2	376
k									
e 1933		2	5	24	77	37	8	1	154
Do			6	24	59	38	11		138
y 1936			4	14	39	36	6		99
Do		1	1	17	65	53	6	1	144
Do			2	20	59	50	8		139
Do			3	13	45	30	5		96
otal		3	23	112	344	244	44	2	772
. Gulf of St. Lawrence:									
vember 1934				3	12	4			19
ember 1935			3	14	39	33	3		92
Do				10	10	15			35
otal			3	27	61	52	3		146
e Bank and vicinity:									
1934		2	4	7	1				14
ember 1934		3	24	48	21	4			100
Do	1	2	28	37	29	3			100
Do	13	13	37	28	17	2			97
Do	1	2	21	57	15	1			97
Do		2	15	19	9				45
ember 1934		5	28	45	26				104
1935		3	21	35	17		1		77
1935				125					125
Do				156					156
1936		3	21	59	12	1			96
Do		2	28	44	19	2	2		97
Do		7	32	61	18	4			122
otal	2	44	259	721	184	17	3		1,230

TABLE A–2.— *Vertebral-number frequencies of haddock from fishing grounds of the northwestern Atlantic and related statistical values*

Area	Number of fish with vertebrae totaling—								n	$\bar{x}$	s
	50	51	52	53	54	55	56	57			
NEW ENGLAND:											
Georges Bank	2	14	185	764	560	87	3	-------	1,615	53.32	0.7965
Passamaquoddy Bay	1	2	25	89	52	7	2	-------	188	53.25	.9099
WESTERN NOVA SCOTIA:											
Digby	1	3	42	169	159	45	2	-------	421	53.48	.8742
Browns Bank	0	6	62	298	283	66	4	-------	719	53.58	.8477
LaHave Bank [1]	-------	-------	4	5	9	1	1	-------	20	53.50	----------
Lockeport	-------	4	19	73	65	32	6	-------	199	53.60	1.0298
CENTRAL NOVA SCOTIA:											
Sambro Bank	-------	-------	7	40	60	20	2	-------	129	53.77	.8338
St. Margaret's Bay	-------	7	72	345	468	260	49	4	1,205	53.83	.9866
EASTERN NOVA SCOTIA:											
Emerald Bank	-------	3	52	318	855	588	88	1	1,905	54.18	.8706
Sable Island Bank	-------	4	32	292	715	504	70	5	1,622	54.18	.8721
Middle Ground	-------	4	20	81	211	151	6	1	474	54.07	.8952
Canso Bank	-------	3	14	66	149	90	12	3	337	54.06	.9651
Quereau Bank	-------	-------	11	42	99	64	16	2	234	54.16	.9849
Ingonish	-------	3	29	141	409	281	49	2	914	54.19	.9033
So. Gulf of St. Lawrence	-------	-------	3	27	61	52	3	-------	146	54.17	.8263
NEWFOUNDLAND:											
St. Pierre Bank and vicinity	2	44	259	721	184	17	3	-------	1,230	52.90	.7648

[1] Statistical values not computed for this small sample.

Age Determination in the Harbour Seal, *Phoca vitulina* L.

RECENT detailed investigations of the life-histories of several arctic and antarctic phocids[1–3] have established the use of incremental layers in the dentine and cementum of canine teeth as accurate indicators of age. Such studies have relied on seasonal differences in the rate and type of deposition for determining the annual nature of the layers, but so far as we are aware, the results obtained have never been validated by examination of teeth from an animal of known age.

Recently, we acquired from Dr. Victor B. Scheffer and Mr. Dale W. Rice, of the U.S. Fish and Wildlife Service, the right upper canine of an old adult male harbour seal from the Seattle Zoo. This animal had been kept in captivity since its capture as a pup, and its age was therefore known to within a few months.

The tooth is illustrated in longitudinal section in Fig. 1, which shows that the bulk of the cementum is confined to the lower half. A thin-ground section in the same plane reveals that the cementum contains many well-defined layers, which are better resolved in the transverse section (Fig. 2) cut at the level marked in Fig. 1. Each layer is about 125μ in width and consists of a wide band of opaque cementum and a narrow translucent band. Examination

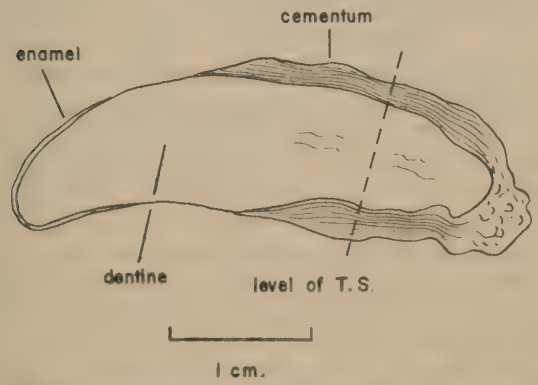

Fig. 1. Diagrammatic longitudinal section of the right upper canine of an old male harbour seal

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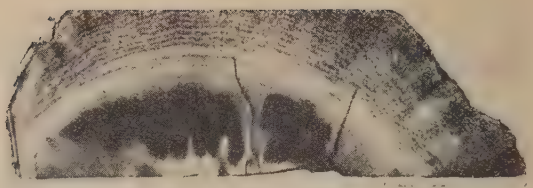

Fig. 2. Half of a transverse section of the same tooth cut at the level shown in the diagram. At least eighteen layers can be counted in the cementum

of other specimens shows that the translucent band is laid down in the spring and early summer and may represent a period of minimal feeding during the breeding period and the moult, as is found in some other phocids[1,3].

Preliminary counts gave the number of cementum layers as 18–20, the lower figure being finally chosen since it was the most certain. After our examination had been made, we learned from Dr. Scheffer that the seal was 19½ years old, a fact which confirmed the annual nature of the cementum layers. There were no clearly marked dentinal layers in this particular tooth, but examination of other canines from both male and female harbour seals has shown that, where dentinal layers are present, they agree in number with those in the cementum.

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Canadian Oceanographic Research in the North East Pacific Ocean

N. P. FOFONOFF and J. D. H. STRICKLAND

Introduction

IN THE LAST DECADE oceanographic research in Canada has been co-ordinated by a Joint Committee on Oceanography, a body consisting of representatives from the main users of oceanographic information, viz., the Royal Canadian Navy, the Fisheries Research Board of Canada, the Defence Research Board, the National Research Council, the Department of Mines and Technical Surveys, and the Department of Transport. Ships are supplied by the Navy and the Fisheries Research Board provides much of the personnel.

A Pacific Oceanographic Group is situated at Nanaimo on Vancouver Island and is concerned with a study of the Pacific Ocean north of about 45° N and east of about 170° W. This area includes the Gulf of Alaska and the coastal regions of British Columbia. Research on the numerous inlets of the British Columbia coast, which present specialized problems, is conducted mainly by the Institute of Oceanography at the University of British Columbia which is a post-graduate training centre for oceanographers.

During and since the last war, defence needs have given an enormous impetus to oceanography throughout the world, as a knowledge of the temperature and density structure of sea water, which directly governs its acoustic properties, is necessary for successful submarine warfare. However, in the broadest sense, oceanographic interests are the marine counterparts of terrestrial meteorology, geology and agriculture and most non-military oceanographic research is in these three fields. The presence of some 3.5 per cent of dissolved salts results in changes to the physical properties of water that are of the greatest importance. A determination of these fundamental properties of sea water as a function of tempera-

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ture, pressure and salt content constitutes a fourth but still sadly neglected branch of marine science. Finally there must be mentioned the important relationship that exists between the behaviour of the atmosphere and ocean, which is now being studied extensively by both meteorologists and oceanographers.

It should be remembered that whereas nearly all the measurements undertaken by oceanographers are comparatively simple to make in a stationary laboratory such measurements when carried out *in situ* at a depth of many thousands of feet from a most unstationary ship become something of an achievement.

Oceanographic Observations

Most of our understanding of the processes taking place in the north Pacific Ocean is based on data collected from survey cruises in which the vertical and horizontal distributions of temperature, salt content, oxygen, and various dissolved constituents are determined. These data are supplemented by observations of currents by direct measurement from an anchored ship or inferred from drift bottles released at various locations in the region of interest.

Two offshore surveys are made each year (mid-summer and mid-winter) together with several cruises in coastal waters. A monitoring program is underway through the year at Ocean Weather Station "Papa" (50° N, 145° W). In addition, daily observations of temperature and salinity are taken at a number of shore stations along the British Columbia coast.

The density of observations in space and time is still extremely low. Over most of the north east Pacific we have to infer the behaviour of the ocean from not more than two observations per year. Large areas of the Pacific have been observed less than once in twenty years. Compared with the meteorologists, who obtain several simultaneous observations of atmospheric conditions each day from a global network of stations, we can hardly be accused of collecting too much data.

Despite the inadequacy of our data, however, we have learned something about the *average* behaviour of the northeast Pacific and something about the variations that occur from year to year.

Ocean Circulation

The basic features of the circulation in the northeast Pacific have been known for many years. The water moves in a slow counter-clockwise gyral around the Gulf of Alaska, often sweeping warm subtropical water northward along the periphery of the Gulf. The mass transport in the gyral is about 20×10^6 metric tons per second with surface velocities of the order of 5 to 10 cm/sec (2 to 5 miles/day).

The circulation is not observed directly however but is inferred from the distribution of density which allows us to see only gradients of velocity. We are able to determine the motion near the surface relative to the deep water, but not the deep water circulation itself. Fortunately, the deep water currents in the North Pacific are very small, even when compared with the deep currents of the Atlantic and Indian Ocean.

The major problems we are confronted with at present is to determine the variations of the northward flow of oceanic water into the Gulf of Alaska and the latitude from which it originates. At times the flow is strongly developed and transports warm water far north of its usual position. We do not know what produces these variations in the circulation from year to year but we suspect that they are induced by gross changes in atmospheric circulation.

Model Studies

Tides present a serious complicating factor in the study of the circulation of water in coastal regions. At any given location tidal fluctuations of currents, temperature, salinity, etc., are apt to be considerably larger than the non-tidal variations. The net circulation and average water characteristics are therefore difficult to obtain. Observations must be averaged over one or more tidal cycles at each location to obtain meaningful results.

As an adequate survey of a large coastal region is beyond our present ship and man power resources, we have attempted to resolve the dilemma by the construction of a hydraulic model of the coastal region. The model (about 100 feet in length) simulates the region between Vancouver Island and the Alaskan Panhandle, including Queen Charlotte Sound, Hecate Strait and Dixon Entrance. It is now in operation at Nanaimo and is used to reproduce tides and to determine the tidal influence on circulation within the region.

Heat Budgets

Neglecting any heat transfer from the earth through to the bottom of the ocean, which is very small, the ocean receives heat by direct conduction from the atmosphere and from solar radiation, the latter being by far the most important source for most of the year in temperature latitudes. Approximately one half of the radiant energy is in the form of visible or ultra violet radiation. The remaining half, in the infra red region of the spectrum, is absorbed by the first few centimeters of the ocean and has little importance other than as a heat source.

Long-term means for incoming solar radiation, over a period of many weeks can be estimated by the use of various empirical formulae involving a knowledge of latitude and cloud cover but for precise work it is

essential to make direct measurements with a pyrheliometer (a form of bolometer). Such instruments are at present installed at Nanaimo, at the University of British Columbia, on the Weathership (50° N, 145° W) and on ocean-going research vessels.

The ocean loses heat to the atmosphere mainly by long-wave radiation, although losses by heat conduction to the atmosphere and by evaporation can be important in the winter. Radiation losses are now measured directly by special bolometers, sensitive to the far infra red but, unfortunately, the losses by conduction and evaporation are only capable of evaluation by empirical means, depending upon a knowledge of the difference between ocean and atmospheric temperatures and upon a knowledge of wind and wave conditions.

It is possible, in principle, to compute complete heat "budgets" of incoming and outgoing heat for a selected body of water if sufficient observations can be made. Such calculations are still comparatively rare but we are attempting to compute heat budgets at several locations near the coast of British Columbia and at the Weathership. By comparing the results of these heat budget computations with the changes of heat content of the water measured by direct thermometry we can obtain important information of broad trends in water movement. Any marked discrepancies between the results obtained by heat budget calculations and by direct measurement must arise from the transport of heat into or out of the region concerned by ocean currents.

Water Budget

The northern regions of the Pacific Ocean receive water from the atmosphere by precipitation and river drainage at an average rate of about 60 cm per year. This influx of fresh water produces a measurable dilution of the surface layer of the ocean to a depth of 200 metres. The diluted layer is found over the entire region north of latitudes 40° N to 45° N and has been used to delinate a region called the sub-Arctic Pacific.

From measurements of salinity of sea water, we can calculate that the fresh water content of the surface layer of the northeastern Pacific Ocean averages about 2%, or the equivalent of about 3.5 metres of fresh water. At the average rate of input, this represents nearly six years accumulation of fresh water. Moreover, as the underlying sea water must be continually mixed into the surface layer to maintain its salt content, the average upward flow into the surface layer must be the order of 20 metres per year. Over the Gulf of Alaska alone, the upward flow amounts to 3×10^6 metric tons per second. The surface water budget is balanced by an escape of surface water southward along the American coast and, to a lesser extent, into the Arctic Ocean through Bering Straits.

Interaction of the Ocean with the Atmosphere

The exchange of heat and water between the atmosphere and ocean represents only part of the interaction between the two fluid bodies. Another important process is the exchange of momentun between the ocean and atmosphere. Winds are important in determining circulation in the ocean. Apart from their direct effect in generating waves and mixing surface layers, winds exert a tangential stress on the surface giving rise to shallow currents. The stress is not uniform over the ocean surface, however, and an accumulation or depletion of surface waters occurs in various regions of the ocean which induces pressure gradients penetrating into the depths of the ocean and giving rise to a flow of deep water.

Computations of these induced currents show that many features of the observed oceanic circulation can be explained on the basis of winds. However, winds are variable both in space and time. It is, therefore, necessary to know the time-dependent response of the ocean. So far most of our knowledge of time dependent processes is based on theoretical studies, which constitute a major part of the research at Nanaimo, but progress is slow because the low frequency response of the ocean is adequately described only by non-linear differential equations and its oscillations cannot be broken down into simple modes.

The Agriculture of the Ocean

There are three main groups of marine organisms that are of importance to fisheries research, the non-planktonic animals, the planktonic animals and the planktonic plants.

At present the average Canadian is only interested in the fish (and shellfish) members of the first of the above groups, although in centuries to come we may have to be more catholic in our tastes.

As most fish have a marked preference for certain ranges of temperature and salt concentration it is the job of the fisheries oceanographer to determine and, if possible, to predict these physical conditions in the sea so that the fisheries biologist can decide their effect on the fish population. In the last few years we have had marked success in predicting the course of the Pacific salmon into the British Columbia river systems as a result of our increasing knowledge of surface water movements off the coast of the province.

The fish we eat feed, in the main, on microscopic or near microscopic animals known as zooplankton. These planktonic animals have little or no net movement with respect to water currents and hence can be concentrated or dispersed by sinking or upwelling of the surface waters of the ocean. The fisheries oceanographer attempts to discover and predict such water movements, as any concentration of zooplankton food will

almost certainly give rise to favourable fishing conditions in the same area. Japanese oceanographers working in the western Pacific, have had marked success with this phase of oceanography. The concentration mechanisms along the west coast of Canada are less pronounced and our knowledge of the complex oceanography of this region is still too scanty for any exact work to be possible at present but an active program to this end is now underway.

The main research program in the field of purely "biological" oceanography by the Pacific Oceanographic Group is concerned with the last group of marine organisms mentioned earlier, the planktonic plants or *phytoplankton*.

As on land, all life in the sea is sustained by the photosynthetic production of plant material, using solar radiation and the abundant supplies of dissolved carbon dioxide found in all sea water. The amount and the rate of production of plant substance must govern the overall food potential of any sea area and differentiate between sea water "deserts" and areas where there are comparatively lush ocean "pastures".

The photosynthetically active region of the solar spectrum coincides roughly with the visual range. All wavelengths are about equally efficient on a quantum basis although red light has, of course, move quanta for a given amount of radiant energy. The quantum efficiency of photosynthesis cannot exceed 25 percent and even half of this efficiency is difficult to realize in practice. In the sea the overall conversion of solar energy will not exceed a few tenths of a percent. Any account of the mechanisms of photosynthesis would be out of place in the present article. In the most general terms it now appears as if the photosynthetic processes of sugar, fat and protein formation are the reverse of the better known respiration and fermentation processes found in living organisms, driven by the photochemical production of high energy phosphorus compounds and complex organic reductants.

Nearly all marine plant life is in the form of microscopic unicellular organisms rarely exceeding 300 microns for the largest dimension and being often as little as 2–5 microns in size. The total production of this plant material in all the oceans of the world is variously estimated to be at least equal to the total terrestial production of plant life (ca. 2×10^{10} tons of organic carbon per year) or perhaps many times greater. Its potential significance as a direct or as an indirect source of protein for an over-populated world need not be stressed.

Plant life is only possible in the surface of any ocean because sufficient solar radiation for photosynthesis to exceed respiration is only found in the top few metres of turbid inshore waters or the top fifty to one hundred metres of clear ocean. Beneath this growth layer, known as the

euphotic zone, plant cells slowly die and sink into the depths. All animal life, from the surface down to abyssal regions of ten thousand metres or more is sustained by the plant growth in this surface skin of water.

The main problems are to measure the amount of phytoplankton in the water, both living cells and detrital material, to measure the rate of photosynthesis of food material by this phytoplankton and to measure and predict the growth factors (mainly nutrients and light) that decide the primary fertility of a given sea area.

The concentrations of all substances to be measured are exceedingly low. The weight or organically combined plant carbon rarely exceeds half a milligram in a litre of sea water and the nutrients responsible for this plant growth (mainly nitrogen and phosphorus and certain metallic growth promotors) are present at concentrations well below a part per million. Advanced microtechniques are required, and a method must be designed to be practical in the hands of a sick man working in a laboratory that may be swaying twenty degrees or more from the horizontal! Despite this, most of the assaying of sea water for plant nutrients has been perfected and in addition we now analyze the suspended plant cells for their protein, carbohydrate, fat and other food potential.

A study of the northeast Pacific reveals at least three main areas of different growth pattern and these patterns doubtless apply to all the oceans of the world. In the open ocean plant growth is vigorous but the crop is comparatively low and its concentration may be limited by continuous heavy grazing by zooplankton. At in-shore locations grazing affects the crop in a more erratic fashion and much of the plant growth there depends on meteorological conditions that alternately allow plant cells to thrive in a stable upper layer or disperse them by vigorous mixing of the surface waters with water at a greater depth. Finally in some large in-shore areas we find that plants strip the nutrients completely from the water in a stable upper layer which is then not recharged with fresh "fertilizer" from deeper waters for prolonged periods. Light is rarely a limiting growth factor except in late autumn and early spring but in these periods the cessation or onset of plant growth is governed by the radiation pattern.

Data Processing

A major obstacle for the oceanographer has been overcome by the utilization of high-speed digital computers to process data. Prior to 1957 roughly half of the effort of the staff at the Nanaimo laboratory went into the computations involved in reducing field observations to a usable form. Since then, all routine computations, not capable of being done

at sea, have been carried out on the Alwac III-E digital computer at the University of British Columbia Computing Centre.

By using high-speed computing aids the results of oceanographic surveys are made available within 5 to 6 weeks of the completion of a cruise. This time may be compared to 6 months required previously for manual processing with essentially the same staff resources.

Conclusion

It is perhaps a cliché to say that we still know next to nothing about the environment of seventy percent of the surface of this planet, but despite the increasing oceanographic activities of the past quarter century the statement is still not much of an exaggeration. The northeast Pacific area is no exception to this despite the efforts of Canadian oceanography, which by any standards have been creditable and which, considering the economic status of Canada as a world power, have been quite exceptional.

Although oceanographic effort throughout the world is increasing at an almost explosive rate it is becoming clear that the magnitude of the distances involved in an area such as the north Pacific Ocean will eventually set a limit to what can be achieved by any foreseeable effort using ships. New techniques must be sought.

In the U.S.A. the submarine is beginning to be used, with obvious advantages in areas of exceptionally poor weather and where there is heavy sea ice. The use of aircraft is another way to extend the range and shorten the time factor in oceanographic observations. Pioneer work in this field is being undertaken at Nanaimo. Water samples and temperature profiles can be obtained from helicopters or even from conventional aircraft with special flying techniques. Much information can be obtained of conditions in the surface layers of the sea by aerial measurements of visible or infra red radiation. Finally it should be possible to use aircraft to receive telemetered information of temperature, salinity and other properties in a water column from a chain of deep anchored monitoring buoys with sensing units on their anchoring cables. This last approach may well point the way to the solution of the outstanding problem facing all oceanographers, the simultaneous description of a large ocean area at closely spaced time intervals.

THE QUEEN'S PRINTER AND CONTROLLER OF STATIONERY

A Substance in Lingcod Liver Oil which prevents Hypercholesterolæmia in Cholesterol-fed Chickens

THE serum cholesterol-level in some animals is elevated above the normal value by the addition of cholesterol to the diet. In certain species, such as the chicken, cholesterol by itself brings about this increase; but in other species, exemplified by the rat, the presence of cholic acid is also required. The degree of hypercholesterolæmia is altered when certain vegetable and fish oils are added to the diet; but the observed effect of any one oil is dependent on the species of animal under investigation. De Groot and Reed[1] showed that cod liver oil and corn oil prevented the increase in plasma cholesterol in rats due to dietary cholesterol and cholic acid. Dam *et al.*[2] investigated the effect of dietary cod liver oil and linseed oil in cholesterol-fed chickens, and found that both oils increased the hypercholesterolæmia caused by the dietary cholesterol. Similar experiments on chickens were performed by Wood and Biely[3] using corn oil, tallow and various fish oils. Corn oil, tallow and herring oil were observed to increase the hypercholesterolæmia; but lingcod liver oil and halibut liver oil completely prevented the appearance of hypercholesterolæmia. Ratfish liver oil, dogfish liver oil and basking shark liver oil were intermediate in effect. In view of the diverse effects of the oils observed in the chicken studies, further investigations were carried out to obtain additional information about the mode of action of the oils. Corn oil, herring oil and lingcod liver oil were chosen for the investigation.

The oils were saponified with ethanolic potassium hydroxide, and the soap solutions were extracted several times with ethyl ether to obtain the unsaponifiable material. The soap solutions were then acidified with hydrochloric acid and the free fatty acids extracted with ethyl ether. The extracts containing the unsaponifiable fractions and the free fatty acids were washed and the solvent removed on a water-bath at 70° C. and under a stream of nitrogen. The oils and their components were added to a diet containing 1 per cent cholesterol. This diet was fed to 2-weeks old New Hampshire chickens for 9 days, after which time blood samples were taken and the serum cholesterol concentrations determined. The oils were fed at a 10 per cent level in the diet and the unsaponifiable substances and the free fatty acids

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Table 1. Effect of Various Oils and Fractions from these Oils on Serum Cholesterol-Levels in Cholesterol-fed Chickens

Addition to the cholesterol-containing diet	Serum cholesterol (mgm./100 ml.)
None	192 ± 12*
Corn oil	784 ± 173
Herring oil	648 ± 133
Lingcod liver oil	130 ± 31
Corn oil fatty acids	793 ± 144
Herring oil fatty acids	618 ± 215
Lingcod liver oil fatty acids	702 ± 158
Corn oil unsaponifiables	193 ± 21
Herring oil unsaponifiables	237 ± 34
Lingcod liver oil unsaponifiables	119 ± 17

* Each value is the mean for four samples of serum ± the standard deviation. Each sample contained the serum from four chickens.

were added in amounts approximating to that obtained from an equivalent amount of oil.

As observed previously, lingcod liver oil prevented the hypercholesterolæmia caused by the dietary cholesterol, and herring oil and corn oil stimulated the rise in serum cholesterol concentration (Table 1). The free fatty acids from all three oils markedly increased the degree of hypercholesterolæmia, whereas the unsaponifiable material from herring oil and corn oil produced only a slight increase or none at all. The unsaponifiable portion of lingcod liver oil, however, completely prevented the rise in serum cholesterol-level.

It was concluded that the ability of lingcod liver oil to prevent hypercholesterolæmia in cholesterol-fed chickens depends on the presence of some compound or compounds in the unsaponifiable material of the oil. At the present moment the active material has not been identified; but the possibility exists that it may be vitamin A or perhaps a sterol-type compound which acts in a similar manner to cholestanol. The latter compound was shown[4] to prevent effectively the appearance of hypercholesterolæmia induced in chickens by cholesterol feeding.

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EXTRAIT
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III. (4)

Developing Effective Use of Hatchery-Reared Atlantic Salmon (*Salmo salar*) in Canada

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Introduction

Principles involved in planting

In Canada, artificial hatching and rearing of Atlantic salmon (*Salmo salar*) were developed on the primary assumptions, (a) that hatcheries assured much higher survival rate of reproductive products than occurred in nature, and (b) that by liberating more of their products in natural waters the hatcheries assured greater catches of adult salmon. These two hypotheses received general acceptance for many years. The hatchery system was built up to the present of supplying some 10 millions of young salmon to streams of the Canadian Atlantic seaboard each year.

During the last 20 to 30 years the validity of these ideas has been subjected to critical appraisement. The modern approach demands both quantitative information on the general value of such hatchery practices, and details as to where and how they might be applied to best advantage.

While continuing to operate its extensive system of hatcheries, the Canadian Government has put increasing emphasis on learning how to improve the preservation of these resources. (1).

Basic techniques used

(a) *Definition of terms.* While the Canadian hatchery system employs a variety of terms to designate young Atlantic salmon of different sizes, the following will be employed in this discussion:—

Underyearlings—fish with the yolk sac absorbed, but less than one year old;

Small parr; large parr—young fish more than one year old but still in the early freshwater stage; "small parr" if under 10 cm in total length, "large parr" if over 10 cm length; (2).

Smolts—young fish just before or during their seaward migration, and showing various degrees of silvery coating as well as other smolt characteristics;

Post-smolts—fish within their first year after passing through a smolt transformation, and which have either assumed a normal sea life or remained in streams and reverted towards parr characteristics.

(b) *Fields of study.* Our researches have involved experimental plantings of hatchery-reared young salmon after liberation in natural stream habitats. River systems used for these experiments have included, in the Province of Nova Scotia: the Moser river; in the Province of New Brunswick: the Miramichi river, the Petitcodiac river and especially its tributary the Pollett river.

(c) *Quantitative measurements.* Counts of plantings either directly or indirectly, were made of young fish when liberated in experimental areas. Estimates of parr derived from results of seining in small sample areas were used to evaluate resulting populations at later stages of stream life. These estimates involved systematic seining, usually with the aid of electro-fishing, and processing of data by standard statistical methods. Two types of estimates were used, one based on repeated mark-and-recapture, the other based on catch per unit of effort. Counts of migrating smolts were made by placing weirs across the lower ends of experimental areas and counting all smolts thus caught.

To compare stream populations derived from hatchery plantings with naturally spawned populations, the initial densities of the latter were measured first as "potential egg deposition", the estimated number of eggs brought into the area by the known number and weight of females present.

Results of Studies of Planting Salmon

1. Supplementary procedures to enhance results of planting

Planting will sometimes give much greater yields of smolts if the liberation of hatchery stock is supplemented by other procedures.

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(a) *Selection of particular stocks.* Of prime importance in this connexion is the matter of "homing." Returns to rivers by planted fish of "foreign" origin, which were marked at the smolt stage, have been relatively few. There are no records, however, of such fish having entered freshwater reaches of streams other than the one in which they were planted.

Adaptability of particular stocks to any extreme physico-chemical characteristics of the system to which they are introduced may also be an important consideration. For example, in the Moser river where some tributaries have low pH values during freshet, some stocks survived flood conditions better than others. With underyearlings of comparable size, held under identical conditions during freshet, 80 % from a less acid stream died as acidity increased below pH 4; 55% from a fairly acid river died and only 20% of native stock died. Mortalities ceased as acidity decreased above pH 4. Other factors such as tolerance of extreme temperature conditions may well have similar importance. (3).

(b) *Control of predators.* Sometimes it may be worth while to forestall heavy losses of planted stock by controlling predators. A prolonged study on the Pollett river showed that control of mergansers (*Mergus merganser americanus*) resulted in smolt production from planted hatchery underyearlings being increased by five times or more. It was concluded that merganser incidence exceeding about one bird for each 10 yards width and 10 miles length of stream during the nine months of open water, would reduce smolt production. Similar benefits from control of mergansers accrue to native populations. (4).

(c) *Establishment of stream capacities.* When planting young salmon which are expected to make additional stream growth before migrating seaward, it would seem a waste to liberate more than are required to saturate the productive capacity of the area. Plantings in the Pollett River showed that the productive rate of this relatively fertile stream, with mergansers controlled, was about five or six smolts per 100 square yards of stream bottom. About 25 to 30 underyearlings planted in summer were sufficient to obtain this rate of production, (although two to three times as many underyearlings and twice as many as the six to eight resulting large parr, could be accommodated by the river). Study of native populations in other Canadian Atlantic streams under varying conditions suggests that this rate of smolt production is widely applicable. But in infertile streams the capacity is somewhat less than half that given above, averaging one to two smolts per 100 square yards of stream bottom. (4).

2. Effective planting procedures

(a) *Dispersal of stocks at planting.* Observati of planted underyearlings indicated that they spr about one mile up and down stream within a weeks of liberation. While some fish moved m farther than this, the more distant ground was effectively utilized. Yearling stocks planted in sum showed similar limited movement during the season. In one experiment, planted parr remained an area originally lacking cover, but to which br and stone obstructions were added, while they not remain in similar adjacent areas where no cover was added. With allowance made for the ab factors, planted fish survived as well to smolt st whether they were poured out at a rate of sev thousand in one small spot or the same number w scattered over 1/2-mile of stream. (4).

(b) *Survival rates from planting to smolt* For summer plantings of underyearlings to give 2-y smolts, the optimum survival rate to smolt st was calculated to be a little over five per c without mergansers controlled, but about fifteen cent. with mergansers controlled. In the latter more fish could be planted, to give five or m times as many smolts as without control. Sim results were obtained for summer plantings yearlings, but the smolts were produced one earlier. (4).

For autumn plantings, survival rates were be being from 20 to 35% to smolt stage when mergan were controlled. Under these circumstances, sim survival rates to smolt stage were observed for l (7 cm) underyearlings migrating mostly 1½ y later, for large (13 cm) yearlings migrating 8 mo later and for large (16 cm) post-smolts migra 8 months later.

Planting smolts which migrate to sea immedia was successful if the fish were (i) selected du the previous autumn to exceed 10 cm total length (ii) liberated during the natural period of s descent. From 25% to 98% of such fish have checked through weirs more than 10 miles d river from the planting site within a month of plantings. When such fish were held over, as smolts, until early autumn they did not mig until the following spring, with interim morta amounting to about 65%.

(c) *Planting to remedy specific deficien* Using techniques based on our research activ it is possible to check the adequacy of local st of young salmon in many streams. There is inc ing evidence that for best results, hatchery st between underyearling and large parr stages sh not be liberated in quantities excessive for fi such natural deficiencies as may exist.

n calculated numbers of well advanced under-
s were added in autumn to a deficient native
tion of similar characteristics the survival rate
h stocks, over 20 months to smolt stage, was
20%. Planting without consideration of existing
can lead either to waste of hatchery products
lack of adequate utilization of rearing areas.
s choices for planting are areas where natural
ng has been deficient or absent. Following the
les enumerated above, deficient local stocks
supplemented with hatchery products at any
tween underyearling and smolt.
me Canadian streams extensive forest spraying
ms using DDT have resulted in serious de-
of particular year-classes of young salmon.
g appears to be the only means of restoring the
l. of such year-classes. Several considerations are
d because insect fauna, especially parr food, is
d: there may be residual poisoning effects for
months: small fish are relatively more suscep-
the poison than large. (5).
implications of hydroelectric development for
an Atlantic salmon streams have only recently
d close attention. Of two basic considerations,
ation of rearing grounds and interference with smolt descent, the latter seems at present more important. Planting smolts below the dams, as done in Sweden, seems one obvious remedy.

Ultimate Value of Planted Stock

Useful information has accumulated on the means of using hatchery stock for increasing smolt runs. Large scale, practical confirmation that such enhanced smolt runs will have commensurate value to the desired fisheries has still to be obtained. Some 125,000 marked smolts were liberated from the Pollett river between 1949 and 1956. Of these about 110,000 were of hatchery origin, mostly planted as underyearlings. Under 15,000 were from native spawned fish. The approximate observed values of these smolts have been as follows: for hatchery-reared stock originating in other rivers 0·02% returned to the planted river, none being recorded in other rivers, and 0·5% were taken in distant commercial fisheries; for native stock in the same stream, 4% have returned to the river of egg origin, none being recorded in other streams and no commercial returns being available as yet.

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STORAGE EFFECTS

ium Ion, Potassium Ion, and Weight nges in Fish Held in Refrigerated Water and Other Solutions

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When sockeye salmon, halibut, herring, lemon sole, and brill were stored in refrigerated sea water for a week, analyses of the flesh revealed a rise in sodium ion and a drop in potassium ion over the natural levels. Weight increases in the fish ranging from 2.4% for salmon to 17.5% for a group of lemon sole were recorded after storage. The outer layer of the flesh of sockeye salmon showed a slow rise in sodium ion and a drop in potassium ion content over the first 48 hours of immersion in chilled sea water, after which time much more rapid changes in the flesh levels of these ions occurred. When solutions were prepared containing sodium and potassium ions at concentrations approximating those calculated for the total tissue water of the fish, the sodium and potassium ions level of the flesh of fish immersed in them remained close to their natural values. It was found that the weight changes of the fish could be prevented by adding 2% of polyvinylpyrrolidone to the immersing solutions.

LDING: SALMON AND HALIBUT in chilled sea water prior to process- a procedure which is rapidly acceptance in the fishing industry northwest Pacific coast. Chilling and reefer tanks have been in- on trollers, seiners, tenders, and , and in canneries in Canada and nited States, including Alaska

rench patent assigned to LeDanois 1-9) covered the holding of fish on sea water or brine, but at −4° C. F.) a temperature which would the fish. Hess reported in 1933 kept twice as well at 30° F., the point of fish muscle, as at 36° to and that a temperature of 30° F. be attained using circulating sea containing ice (*7*). The engi- g, bacteriological, chemical, and nical aspects of holding fish in rated sea water have been the sub- considerable study at the Techno- Station over the past several years. concentrations of sodium and sium ions in the flesh of living fish little from those prevailing in d tissues (*11*). Sodium and po- ium ions in sea water are present at ximately 20 and 0.07 times, respec- the concentration of these ions in flesh. Living fish maintain these ntration gradients by expending energy. Dead fish, on the other hand, if maintained in sea water, tend to equilibrate with their surroundings. The sodium ion concentration of the flesh rises, the potassium ion drops (*13*), and the fish increase in weight (*2*).

In this paper the sodium and potassium ions and the weight changes in fish stored in refrigerated sea water are recorded for various species. The effect of varying the salt concentrations and osmotic pressure of the immersing solutions on the extent of the changes observed is also reported.

Methods

Unless otherwise indicated, fish were cleaned and then sampled according to a slight modification of the official method (*7*). Samples of homogenized fish were dissolved in nitric acid and analyzed for sodium and potassium ions using a flame photometer attachment for a Beckman DU spectrophotometer. Details of the sampling and analytical procedures used have been described (*11*). Two methods were employed to maintain fish in refrigerated sea water or other salt solutions for the desired length of time. In one case a laboratory scale model of equipment designed for use on a fishing boat was employed. Fish were held in the immersing solution in a plywood box painted on the inside with Terecote, a Thiokol-type water-resistant coating (Technical Research Co., Seattle, Wash.). The immersing solution was continuously circulated at a rate of 4.5 gallons (U. S.) per hour through the box and over pipes containing refrigerant in an outside chilling unit. The temperature of the immersing solution was maintained at 30° ± 0.5° F. When the effect of a number of different immersing solutions on fish was being compared, 5-gallon plastic pails equipped with stirrers were used and the experiments were conducted in a refrigerated room maintained at approximately 32° F.

The ratio of fish to immersing solution was maintained as closely as possible to one part by weight of fish to four of solution. Chlortetracycline was added as an antibacterial agent to all immersing solutions at a level of 10 p.p.m. Artificial rather than natural sea water was used throughout this study because the salt concentrations were more readily reproducible than with the natural sea water available to this laboratory. The artificial sea water had the following composition (*10*) in grams per kg.: NaCl, 23.476; $NaSO_4$, 3.917; $NaHCO_3$, 0.192; KCl, 0.664; KBr, 0.096; $MgCl_2$, 4.981; $CaCl_2$, 1.102; $SrCl_2$, 0.024; H_3BO_3, 0.026. Half-strength sea water was prepared by diluting the artificial

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sea water with an equal volume of fresh water.

Results

The effect on the sodium and potassium ion content of the flesh of storing eviscerated coho salmon (*Oncorhynchus kisutch*) in refrigerated sea water for periods up to 4 weeks has been reported (*13*).

In the present study, the earlier observations were extended in the following manner. Sockeye salmon (*Oncorhynchus nerka*) were used in place of coho. Artificial rather than natural sea water was employed as the immersing solution to provide more readily reproducible salt concentrations. As sea water along the coastline is frequently diluted with fresh water, particularly near points where rivers discharge into the sea, the artificial sea water was tested at both full and half strength. As fish are frequently eviscerated immediately after capture, a comparison was made of the extent of the changes occurring in whole and eviscerated fish. Weight changes in the fish were also determined. The results are recorded in Table I. For this experiment, sockeye salmon freshly caught in a gill net at the mouth of the Fraser River were quickly transported to the laboratory and immediately immersed in previously prepared solutions. Less than 4 to 6 hours elapsed between the time of capture of the fish and the beginning of the experiment.

The results show (Table I) that half-strength sea water raised the sodium ion level of the flesh of the fish approximately half as high as full-strength sea water, but produced little difference in the potassium ion concentration. Although the eviscerated fish presented a greater surface exposed to the salt solution than did the whole fish, surprisingly little difference was observed in the sodium and potassium ion concentrations of the flesh from the two groups. Fish held in full-strength sea water tended to gain more weight than those in the half-strength solution, while the increase observed in eviscerated fish exceeded that in whole ones.

In the experiment just described, the first analyses were made after immersion of the fish for 1 week in the sea-water solutions. In previous experiments with coho salmon in which fish were immersed for short intervals in sea water, the natural levels of sodium and potassium ions were maintained in the fish for a period of approximately 24 hours, after which time a rather abrupt rise in sodium ion and fall in potassium ion occurred (*13*).

To determine whether a similar phenomenon could be observed in another species, an experiment was conducted using sockeye salmon. In an effort to cast further light on the nature of the phenomenon, the flesh in a 1-inch slice taken through the center section of the fish was divided into inner and outer layers. The inner layer represented the flesh from the backbone to a point midway between the backbone and the outer surface of the fish, while the outer layer was made up of the flesh from the mid-point to the outer surface. The layers were rendered skin- and bone-free and then homogenized. The flesh from a second 1-inch slice through the fish taken at a point adjacent to the first was pooled in the usual manner for analysis. For this experiment sockeye salmon were caught in gill nets at the mouth of the Fraser River and transported immediately to the laboratory. Approximately 4 hours elapsed from the time of capture of the fish until the whole fish were immersed in the experimen water tank. At each 6-hour in three fish were removed from th for sampling and analysis. The obtained are recorded in Figure 1. outer layer of flesh showed a slow sodium ion concentration and potassium ion which was eviden the earliest analyses. After ap mately 72 hours these changes be take place much more rapidly change occurred in the ion levels inner layer of flesh during the fi hour period. As can be seen, t crease in the rate of change of t concentrations in the outer lay curred after changes in sodium potassium ion levels had begun t place in the inner layer of flesh might be expected, values obtained

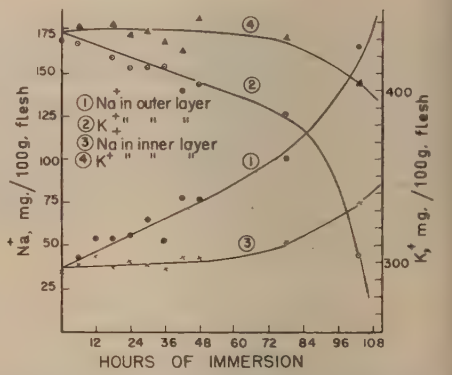

Figure 1. Na^+ and K^+ changes in inner and outer layers of muscle tissue of sockeye salmon at various intervals after immersion of the fish in full strength refrigerated sea water

Each recorded point represents average value for three fish

Table I. Sodium and Potassium Ions and Weight Changes in Soc Salmon Held Whole and Eviscerated in Refrigerated Sea Water

Sea Water Concentration	Immersion Time, Weeks	Mg./100 G.[b] Na^+	Mg./100 G.[b] K^+	Wt. Increase %
Control (not immersed)	...	33.5 ± 1.1	411 ± 15	
		Whole fish		
Full strength	1	277 ± 13	276 ± 5	2.98 ± 0.
	2	422 ± 18	198 ± 6	4.33 ± 1.
Half strength	1	149 ± 12	265 ± 9	2.38 ± 0.
	2	221 ± 20	201 ± 17	3.45 ± 0.
		Eviscerated Fish		
Full strength	1	297 ± 38	272 ± 19	4.63 ± 1.
	2	487 ± 26	193 ± 19	8.16 ± 1.
Half strength	1	178 ± 29	257 ± 34	3.89 ± 0.
	2	250 ± 21	178 ± 25	5.60 ± 1.

[a] Of entire fish.
[b] Based on wet weight of muscle tissue. Each value represents average and av deviation of four fish.

ample representing a composite ner and outer layers, were ap- tely the average of the two sets shown

itial slow rise in sodium ion and tant fall in potassium ion in the ver of the flesh (Figure 1) may ve been due to equilibration of mersing solution with the extra- fluid of the tissue, while the more hanges occurring subsequently present equilibration with intra- fluid. Studies with much sections of tissue would be re- o test this hypothesis further.

mation on ion and weight changes mersed in refrigerated sea water ar been restricted to studies on species of salmon. As a step extending these observations to other commercially important of fish, an experiment was con- with halibut (*Hippoglossus steno-* For this purpose, halibut rang- weight from 10 to 20 pounds were at sea and packed in ice for trans- the laboratory. The experiment was begun within 24 hours of the time of capture of the fish. In this study a slice 1 inch wide was taken from the center section of the fish and divided into inner and outer layers. As in the previous salmon experiment, the inner layer represented the flesh from the backbone to a point midway between the backbone and the outer surface, while the outer layer was composed of flesh from this mid-point to the outer surface. The layers were freed of skin and bone and then homogenized. The results (Table II) show that after 1 week in half-strength sea water, the inner layer of flesh of the halibut was almost unchanged with respect to sodium and potassium ion concentration, while the values for the outer layer had changed very appreciably. After 2 weeks the two layers had still not equilibrated. The weight increases recorded after 1 and 2 weeks are slightly higher than for sockeye salmon held under comparable conditions.

It was of interest to know whether sea water could be modified or solutions devised which would minimize or eliminate the changes observed in the preceding experiments. To investigate this possibility, smaller species of fish were selected to enable a reasonable number of different solutions to be tested at the same time with the facilities available.

In Table III are recorded the results obtained when herring were immersed for 1 week in various solutions. For experiments with herring the fish were obtained from seine boats as soon as possible after capture, which was usually within 24 to 36 hours. January-caught herring were used. The whole herring were weighed individually before being immersed in the various solutions. In the case of immersion in full-strength sea water the results show (Table III) that the uptake of sodium ion was somewhat higher for herring than had been observed for sockeye salmon under comparable conditions (Table I). The potassium ion loss for herring was a little less and the weight changes were slightly higher.

As the sodium ion uptake by and the potassium ion loss from the cells could possibly be due to exchange (*6*), it seemed not unlikely that if potassium ion loss could be prevented, sodium ion uptake might not occur. Accordingly potassium ion was added to the sea water until the concentration equaled that calculated for the potassium ion concentration of the total tissue water in the flesh of the fish. Although this concentration of potassium ion in the immersing fluid not only maintained but somewhat increased the potassium ion concentration in the flesh, it failed to prevent the uptake of sodium ion (Table III). Similar results were obtained when potassium ion was added to half-strength sea water.

From a knowledge of the moisture content of the flesh and its content of sodium and potassium ions, one can calculate the concentration of sodium and potassium ions in the total tissue water. If the immersing fluid contained sodium and potassium ions in this same concentration, it should be possible to maintain the flesh levels of these ions approximately constant. Solution 5 in Table III was prepared to test this possibility. The total tissue water of an average herring was calculated to contain 1.09 grams of sodium ion and 6.44 grams of potassium ion per liter. These levels were provided in the immersing solution using appropriate amounts of sodium chloride and potassium chloride. Although some changes did occur as a result of immersion of the fish in this solution, a reasonable approximation of the starting values was maintained (Table III).

A series of solutions was tested using lemon sole (*Parophrys vetulus*) as the experimental fish. This species was chosen because it is a small flat fish with a high ratio of surface area to body weight. In addition, as it never accumulates any

ii. Sodium and Potassium Ion Changes in Inner and Outer Layers of e Tissue of Halibut and Weight Increases in Fish Preserved in Half-Strength Refrigerated Sea Water

Portion of Tissue Analyzed	Mg./100 G. Na^+	Mg./100 G. K^+	Wt. Increase,[a] %
Inner layer	45.6 ± 6.1	510 ± 9	
Outer layer	47.7 ± 5.1	493 ± 15	
Average	46.7 ± 5.6	501 ± 12	
Inner layer	67.4 ± 5.1	501 ± 15	3.7 ± 0.7
Outer layer	149.3 ± 22.9	389 ± 17	
Average	108.4 ± 14	445 ± 16	
Inner layer	137.4 ± 19.1	413 ± 22	5.1 ± 0.9
Outer layer	210.8 ± 19.1	302 ± 23	
Average	174.1 ± 19.1	358 ± 23	

entire fish
ed on wet weight of muscle tissue. Each value represents average and average on of four fish.

iii. Changes in the Sodium and Potassium Ion Content of the e Tissue and in the Weight of Herring Held for 1 Week in Various Immersing Solutions

Composition of Solution[a]	Mg./100 G.[b] Na^+	Mg./100 G.[b] K^+	Wt. Increase, %
Control, not immersed	68 4 ± 1.9	446 ± 8	
Sea water (full strength)	365 ± 20	305 ± 23	3.4 ± 0.6
Solution 1 + K^+	412 ± 21	522 ± 3	3.8 ± 0 6
Sea water (half strength)	227 ± 30	267 ± 10	4.3 ± 1.0
Solution 3 + K^+	229 ± 9	554 ± 7	5 3 ± 0.6
Na^+ and K^+ to maintain flesh level constant	79 ± 5	542 ± 19	7.7 ± 0.8

lution 2. Sea water + 11.61 grams/l. of KCl. Solution 4. Half-strength sea + 11.97 grams/l. KCl. Solution 5. 2.76 grams/l. of NaCl + 12.33 grams/l. of

ed on wet weight of muscle tissue. Each value represents average and average on of six fish.

appreciable levels of fat as compared to herring or salmon, it can be considered also as representative of the nonfatty fish. When lemon sole were immersed in half-strength sea water for 1 week (Table IV), the sodium ion uptake and potassium ion loss greatly exceeded those for sockeye salmon and herring held under comparable conditions (Tables III and VI) and the weight increase, 17.5%, far exceeded that for any of the other species of fish examined. A solution of sodium and potassium chlorides designed to maintain the flesh levels constant again came close to achieving the desired effect (solution 2). Such a solution can be readily prepared from sea water by mixing 1 part of sea water with 9 parts of fresh water and adding potassium chloride. When tested (solution 3) it caused some rise in sodium ion, but maintained the potassium ion level in the flesh of the lemon sole close to the value at the start of the experiment.

Little difficulty is experienced in preparing solutions which will maintain the level of sodium and potassium ions in the flesh at or near their natural values. Fish immersed in such solutions, however, usually gained more weight than when stored in sea water. As no significant net uptake of salt was involved when these solutions were used, the weight increase observed could only be due to uptake of water by the flesh. Water uptake would be expected to occur if the osmotic pressure of the immersing solution was lower than that of the tissue fluids of the fish. It was therefore desirable to find an agent which would be sufficiently soluble to provide the required osmotic pressure in the solution but be impermeable to the tissue membranes of the fish. Such an agent would be expected to have properties similar to those of the compounds employed to maintain osmotic pressure in plasma substitutes (*4*). A compound used for the latter purpose, polyvinylpyrrolidone (PVP) was tested to determine its capacity to prevent weight increases in lemon sole (Table V). Only 2% of this compound prevented water uptake from a solution of sodium and potassium chlorides and from full and half-strength sea water. Separate experiments showed that higher concentrations of polyvinylpyrrolidone actually caused the fish to lose weight.

Discussion

To compare the extent of the changes occurring in various species of fish immersed in refrigerated sea water, the results for fish held under comparable conditions for 1 week are summarized in Table VI. The smallest changes in sodium and potassium ions occurred in halibut. As the changes recorded represent the average for the flesh of the fish taken in cross section, the larger the fish the less the change that would be expected in such values, if insufficient time had elapsed for complete equilibration of the flesh with the immersing solution. As Table II showed, the inner layer of the flesh of halibut had not reached the sodium ion content of the outer layer at the end even of the second week of immersion.

For fish of the same weight one might expect the greatest changes to occur with fish having the highest ratio of surface area to body weight. Although this would appear to be borne out by a c parison of the changes in herring and first group of lemon sole (Table VI second group of lemon sole showe weight increase similar to that in herring and much less than in the group of fish. The first group of le sole were caught in March and conta no visible milt or roe, while the se group were captured in November contained well developed gonads. marked differences in the physiolog state of the living fish could well hav influence on the extent of changes curring in storage. It may be of sig cance that the skin of herring (*12*) salmon (*15*, *17*) has been observe

Table IV. Variations in Sodium and Potassium Ion Content of Fl and Weight Changes in Lemon Sole Held for 1 Week in Solutions Various Compositions

Solution, No.	Composition of Solution[a]	Mg./100 G.[b] Na^+	Mg./100 G.[b] K^+	Wt. Increa %
...	Control (not immersed)	89 ± 1.0	387 ± 2	...
1	Sea water (half strength)	311 ± 9	138 ± 4	17.5 ± 1
2	Na^+ and K^+ to maintain flesh level constant	113 ± 1.4	405 ± 3	18.2 ± 1
3	1 part sea water + 9 parts fresh water + K^+ to maintain flesh level constant	115 ± 2	378 ± 18	22.9 ± 4

[a] Solution 2. 2.35 grams of NaCl and 10.24 grams of KCl per l. Solution 3. Indic amounts of sea water + 10.3 grams/l. of KCl.
[b] See Table III.

Table V. Capacity of Polyvinylpyrrolidone to Prevent Weight Increa in Lemon Sole Immersed in Salt Solutions

Solution, No.	Salt Composition of Immersing Solution[a]	Polyvinyl-pyrrolidone Addition, %	Wt. Change[b]
1	Na^+ and K^+ to maintain flesh level constant	0	+ 8.5 ± 1.3
2	Same as solution 1	2	+ 0.7 ± 0.5
3	Sea water (half strength)	0	+ 7.8 ± 1.3
4	Sea water (half strength)	2	+ 0.1 ± 1.4
5	Sea water (full strength)	0	+11.5 ± 2.4
6	Sea water (full strength)	2	+ 0.8 ± 0.6

[a] Solution. 1. 2.37 grams of NaCl and 7.9 grams of KCl per l.
[b] Each value represents average and average deviation of 10 fish.

Table VI. Sodium and Potassium Ions and Weight Changes in Var Species of Fish Held 1 Week in Half-Strength Refrigerated Sea Wat

Species	Weight, Lb.	Change Recorded,[a] Mg./100 G. Na^+	Change Recorded,[a] Mg./100 G. K^+	Wt. Increase, %
Sockeye salmon	5.29 ± 0.23	+115	−146	2.4 ± 0.6
Halibut	15.2 ± 1.7	+ 61	− 56	3.7 ± 0.7
Herring	0.33 ± 0.04	+159	−179	4.3 ± 1.3
Lemon sole				
1	0.65 ± 0.08	+222	−249	17.5 ± 1.9
2	1.0 ± 0.05	+273	−266	5.5 ± 1.7
Brill (*Eopsetta jordani*)	1.7 ± 0.27	+223	−213	7.8 ± 1.3

[a] Change recorded in Na^+ and K^+ levels represents difference between amounts pr before and after immersion for 1 week in half-strength sea water.

:e a greater proportion of the
eight of the fish just prior to
g than at other stages in their
maturation.

lation to the other fish (Table
. the herring and salmon appear
increased in weight less than
ave been expected from the ex-
he changes in their sodium and
m levels. Whether this means
osmotic pressure of the intra-
fluids is lower in these fish than
hers or that the higher fat content
wo species specifically impeded
ake of water cannot be deter-
rom the data.

act that storage of fish in refrig-
ed water produces an uneven
ion of sodium ion in the flesh
that analyses based on the
method of sampling (*1*) in this
vide insufficient information for
g purposes. A situation could
ibly arise wherein the outer layer
a fish contained an unpalatable
sodium ion, the inner layer re-
almost unchanged, while a value
on the cross-sectional sample
in the official method indicated
sodium ion within the limits of
lity.

have been many studies in
hydration of mammalian tissues
n observed in salt solutions iso-
with blood and tissue fluids, and
are conflicting explanations to
for the phenomenon (*8*, *16*, *18*).

In this study, where the ion concentrations of the immersing solution were adjusted to maintain the flesh levels constant, water uptake must have been due primarily to the hypertonicity of the intracellular fluids of the flesh. This was further substantiated by the observation that a nondiffusible substance capable of increasing the osmotic pressure of the immersing solution prevented water uptake by the fish.

Polyvinylpyrrolidone is a chemically inert, hydrophilic, water-soluble macromolecule essentially nontoxic for humans by all routes of administration. The compound is widely used in the formulation of pharmaceuticals and has been recommended for use as a beverage clarifier (*5*).

In the practical application of the results reported here it would be feasible to premix and package sodium chloride, potassium chloride, and polyvinylpyrrolidone for convenient addition to tanks of fresh water on fishing vessels or in shore plants.

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SOME FURTHER RECORDS OF PELAGIC POLYCHAETA FROM THE NORTHEAST PACIFIC NORTH OF LATITUDE 40° N. AND EAST OF LONGITUDE 175° W., TOGETHER WITH RECORDS OF SIPHONOPHORA, MOLLUSCA, AND TUNICATA FROM THE SAME REGION[1]

E. Berkeley and C. Berkeley

Abstract

Seventeen species of pelagic Polychaeta from the northeastern Pacific are recorded. Of these, five (*Sphaerosyllis pirifera* Claparède, *Dorvillea kefersteini* McIntosh, *Spiophanes cirrata* Sars, *Magelona* sp., and *Flabelligera affinis* Sars) are species which, whilst not exclusively pelagic, are known to swim at some stage, particularly as larvae. One record (*Pedinosoma curtum* Reibisch) is new to the Pacific Ocean, two (*Pontodora pelagica* Greef and *Callizona nasuta* Greef) are new to the northeastern Pacific region, and four (*Pelagobia longicirrata* Greef, *Lopadorhynchus uncinatus* Fauvel, *Sagitella kowalewskii* Wagner, and *Callizona angelini* (Kinberg)) are records of northerly extensions of distribution. The first observation of a larval Cossura (probably of *C. longocirrata* Webster and Benedict) is recorded. *Poeobius meseres* Heath is recorded from a number of stations and the classification of Poeobiidae is discussed.

Eight species of Siphonophora are listed, all of which constitute new records for the region covered by the present collections. Six species of Mollusca, comprising five Pteropoda and one Heteropoda, are recorded, none of which have been identified previously in northeastern Pacific plankton. Three of the genera of Pteropoda (*Anopsia*, *Thliptodon*, and *Cliopsis*) are characteristic of warmer seas, the other two, and the heteropod, are widely distributed. Of the two species of Tunicata listed one is new to the northeastern Pacific.

Introduction

In 1957 (6) we recorded a number of pelagic polychaetes which had been collected in plankton samples by the vessels engaged in the survey activities of the Fisheries Research Board of Canada in the northeast Pacific during 1955 and 1956. The present paper records species taken in the course of similar activities during 1957, 1958, and 1959, together with notes on a few others which, though not usually regarded as pelagic, have been found free-swimming in the region in which the collections were made.* Several of the species mentioned herein were already recorded in 1957, but from single, or few, specimens. Others (such as *Tomopteris septentrionalis* Quatrefages and *Pelagohelmis tenuis* (Apstein)), which are of very general occurrence, are now omitted.

During the years we have been examining specimens from these plankton collections we have encountered a number of animals belonging to groups other than the Polychaeta. These we have sent to specialists in the several fields for identification and we now append their findings in respect of the Siphonophora, Mollusca, and Tunicata.

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*For chart of this region see reference 6.

Grateful acknowledgment is due to Mr. R. LeBrasseur, of the Fisheries Research Board of Canada, Nanaimo Biological Station, for the care exercised in the course of his sorting of the original plankton material, in separating the samples on which the identifications now recorded have been based.

Polychaeta

Phyllodocidae

Pelagobia longicirrata Greef. Fauvel (12), Ushakov (36)

47° 00′ N., 136° 07′ W., 150–0 m, one specimen
50° 07′ N., 144° 50′ W., 150–0 m, one specimen
50° 05′ N., 145° 20′ W., 150–0 m, one specimen
50° 01′ N., 145° 02′ W., 150–0 m, one specimen
43° 42′ N., 136° 00′ W., 300–200 m, four specimens
45° 02′ N., 147° 06′ W., 150–0 m, one specimen
50° 00′ N., 145° 00′ W., 150–0 m, several specimens

The present records extend the distribution of this species in the northeast Pacific northwards, that of Treadwell (35, as *P. vigiueri*), at 40° N., being the most northerly previous one. In the northwest Pacific Ushakov (37, map 1) indicates its occurrence at about 55° N. and he has also recorded it in the north polar region (38). It is widely distributed in the Atlantic, in the Mediterranean and Indian oceans, and in the Antarctic. It may, therefore, be regarded as cosmopolitan.

This species and *P. vigiueri* Gravier have been confused in the literature. They are now regarded as synonymous (37).

Pontodora pelagica Greef. Fauvel (12), Ushakov (37)

50° 00′ N., 145° 00′ W., 1250–0 m, five anterior, one posterior, one median portion

The specimens agree with Fauvel's (12) and Ushakov's (37) descriptions. The cirri are not easily distinguished and a few of them are lost in some of the specimens. The orange spotting (due, apparently, to an oily secretion) is very conspicuous in some of them.

The species has not been described previously from the northeast Pacific and there appears to be no record of it anywhere in the north Pacific north of about 40° N. (37). Ushakov (37) records several instances of its occurrence off the coast of Japan and from the Arctic Ocean between 75° N. and 85° N. (38). He considers *Epitoka pelagica* Treadwell is a synonym, a conclusion with which we agree. All other records from the Pacific are from the southern hemisphere (35). The species is known from the Mediterranean and, in the Atlantic, from the Canary Islands.

Lopadorhynchus uncinatus Fauvel. Fauvel (12), Wesenberg-Lund (39), Dales (11)

54° 30′ N., 152° 00′ W., 150–0 m, one specimen

A single complete specimen a little more than 1 mm long, probably larval, agrees completely with the characters of the adult as given by the above

authors. Dales' record from about 38° N. (11, Fig. 6) is the most northerly in the northeast Pacific hitherto. It is now recorded considerably further north. Treadwell (35, as *L. brevis* and *L. nans*) records it from off Japan and from numerous stations in the South Pacific. It is common in the Mediterranean and is recorded from the north and south Atlantic.

Pedinosoma curtum Reibisch. Reibisch (31), Fauvel (12)

53° 32′ N., 151° 57′ W., 150–0 m, one specimen

A single specimen 1 mm long agrees with the descriptions of Reibisch and Fauvel. The species has not been recorded previously from the Pacific. Earlier records are mainly from the southern Atlantic between the equator and 20° N., the most northerly point reached being in the neighborhood of the Azores (31, Taf. 7), and in the Mediterranean.

Phalocrophorus pictus Greef. Fauvel (12)

50° 28′ N., 131° 11′ W., 150–0 m, one specimen
43° 42′ N., 136° 00′ W., 150–0 m, two specimens, one with extended jaws
50° 01′ N., 145° 02′ W. (approximate), one specimen

Our previous record of this species (6) remains the farthest north in the north Pacific.

Typhloscolecidae

Travisiopsis lobifera Levinsen. Fauvel (12)

51° 00′ N., 130° 00′ W., 20-m tow, three large specimens
50° 00′ N., 145° 00′ W., 150–0 m, three specimens
47° 30′ N., 138° 54′ W., surface, one specimen

This species was recorded from a single specimen in 1957 (6). It is now found at about the same latitude, but further east.

Typhloscolex mülleri Busch. Fauvel (12)

50° 55′ N., 129° 08′ W. (approximate), 50-m tow, one specimen
50° 27′ N., 132° 11′ W., 150–0 m, one specimen
52° 02′ N., 136° 00′ W., 50-m tow, two specimens
55° 03′ N., 149° 54′ W., 150–0 m, one specimen
54° 30′ N., 152° 00′ W., 150–0 m, one specimen

In addition to the above the species has been taken from four other stations in the map area for which bearings are not available. It was reported in 1957 (6) from three stations and would thus appear to be fairly common.

Sagitella kowalewskii Wagner. Fauvel (12)

50° 01′ N., 145° 02′ W., 1200–0 m, one specimen
43° 42′ N., 136° 00′ W., 300–200 m, four specimens
50° 01′ N., 145° 02′ W., 150–0 m, one specimen

This species was recorded by one of us (E. B.) off the west coast of Vancouver Island at about 49° N. in 1930 (2). This remained the most northerly point in the northeast Pacific from which it was known until the present

records. It was, however, previously found at a slightly more northerly station in the northwest Pacific by Ushakov (37, map 4). It is more generally known from warmer seas.

Syllidae

Sphaerosyllis pirifera Claparède. Fauvel (12)

57° 32′ N., 151° 42′ W., 150–0 m, one specimen

This species, previously recorded from both coasts of Vancouver Island (3), has not, hitherto, been taken as a planktont. Fauvel (12) mentions swimming forms with long natatory setae in the male. These characterize the present specimen. It is interesting to find it so far from coastal waters.

Autolytus varius Treadwell. *Sacconereis*. E. and C. Berkeley (3)

53° 53′ N., 148° 00′ W., 150–0 m, four specimens

This species is a well-known offshore planktont in the Nanaimo region in the *Sacconereis* phase, but has not previously been found so far at sea. The specimens are typical.

Alciopidae

Callizona angelini (Kinberg). Fauvel (12)

50° 00′ N., 145° 00′ W., 150–0 m, two specimens
52° 30′ N., 142° 00′ W., surface at night, one specimen
53° 17′ N., 143° 53′ W., 25-m tow, one specimen
50° 00′ N., 145° 00′ W., 150–0 m, three specimens
55° 40′ N., 151° 10′ W., surface, one specimen

The earliest records of this species in the northeast Pacific were off the east coast of Vancouver Island at about 50° N. lat. and 125° W. long. (2). In 1957 (6) we listed a second one at about the same latitude, but as far west as 169° W. The present records are from stations intermediary in longitude between these extremes, thus extending the distribution across a considerable region of the width of the map area at about 50° N. lat. The northerly range is now extended to 55° N. lat.

The species is recorded off Japan and, widely, between 20° N. lat. and 20° S. lat. by Treadwell (35, as *Rhynchonerella pycnocera*). It is fairly common in the Atlantic (39).

Callizona nasuta Greef. Fauvel (12), Wesenberg-Lund (39)

48° 32′ N., 126° 09′ W., 50–0 m, one specimen
45° 00′ N., 147° 00′ W., 150–0 m, one anterior fragment
54° 30′ N., 152° 00′ W., 150–0 m, one anterior fragment

This species has not been recorded previously from the northeast Pacific. It is, however, known off the coast of Japan (37). The present specimens are typical and are readily recognizable even in the absence of the posterior regions. There is only one heavy and simple acicular seta in any of the anterior setigers. This, the projection of the central part of the prostomium in front of the eyes, and the narrow, sharply pointed, dorsal cirri are quite characteristic. It is known from the Atlantic and Mediterranean.

Tomopteridae

Tomopteris (*Johnstonella*) *renata*. E. and C. Berkeley (3)

50° 26′ N., 151° 24′ W., 150–0 m, one incomplete specimen
50° 01′ N., 145° 02′ W., 1200–0 m, one incomplete specimen
50° 01′ N., 145° 02′ W., 150–0 m, three good specimens
50° 01′ N., 145° 02′ W., 150–0 m, one specimen
55° 00′ N., 150° 00′ W., 60-m tow, one good specimen

In our 1957 paper (6) we commented on the absence of this species from the plankton samples and suggested that it might be an inshore form in the northeast Pacific, though it had been taken in open waters from regions farther west (36). The above records show that this surmise was erroneous. The characters differentiating the present species from *T. pacifica* Izuka, which we pointed out in our previous paper (6), are well exemplified in the present specimens. The last one listed is the largest yet known (45 mm long, excluding the tail).

Eunicidae

Dorvillea kefersteini McIntosh. McIntosh (27), Fauvel (12)

50° 55′ N., 129° 08′ W., 50–0 m, one specimen

The single small specimen (about 5 mm long as preserved) agrees with this species except in that the antennae are rather shorter and thicker than described (cf. ref. 12, Fig. 177 *m*). This may well be due to excessive contraction. The dorsal cirri lack both cirrophores and acicula, a character it shares with *D. gracilis* Hartman, but it differs from that species in having the palps non-articulate, except at the tips, and parapodia with much longer setal lobes.

D. kefersteini is not a true pelagic species, though McIntosh (27) records its ability to swim. It has not been recorded previously from the west coast of North America.

Spionidae

Spiophanes cirrata Sars. E. and C. Berkeley (4)

48° 33′ N., 123° 30′ W., surface, eight larval specimens

The specimens vary in length from 1 to 2 mm. The longer individuals are at an advanced larval stage consisting of 20 segments. Most of the swimming setae have been shed. These larvae are readily differentiated from those of *S. bombyx* (Claparède), the only other, and much less common, species known in the region, by the absence of prostomial "horns" and the presence of tridentate crotchets in posterior setigers.

The adults of this species are mud dwellers. The larvae have not been described, but a very complete account of those of a very nearly allied form, *S. kröyeri* Grube, is given by Hannerz (15). The present specimens agree closely with this account. It would not be anticipated that the single character differentiating *S. cirrata* from *S. kröyeri* (the presence of "soies bacillaires" in the former, but not in the latter) would be apparent in the larvae. Larvae of *S. kröyeri* are said to be common at particular seasons in the plankton in Gullmar Fjord, Sweden (15).

Magelonidae

Magelona sp. E. and C. Berkeley (4)

44° 9′ N., 130° 53′ W., 100–0 m, four larvae

51° 34′ N., 133° 24′ W., 50–0 m, one larva

Most of the specimens are less than 2 mm long. Whilst their generic identity can be recognized from the long papillated palps the adult characters are not sufficiently developed for specific determination. Adults of two species, *M. japonica* Okuda and *M. pitelkai* Hartman, are known from the region. Assuming the larvae to belong to one or other of those species, *M. pitelkai* seems to be the more probable, judging by the form of the prostomium and of the special setae of the ninth setiger. The larvae of neither *M. japonica* nor *M. pitelkai* have been recorded. Those of other species of *Magelona* are known to occur in the plankton (33, 22).

Cirratulidae

Cossura sp. (probably *C. longocirrata* Webster and Benedict). E. and C. Berkeley (5)

51° 34′ N., 133° 24′ W., 50–0 m, one larva

The specimen is about 2 mm long and consists of about 22 segments. Measurements are difficult owing to its curled condition. It is incomplete posteriorly. The single long median cirrus (branchia) is characteristic of the genus, but specific identification is impossible. It is probably *C. longocirrata* Webster and Benedict, since this is the only Cossura recorded from the northwest coast of North America north of California (5). Two other species are known from California, *C. candida* Hartman and *C. pygodactylata* Jones. The former is excluded in the present instance since the branchial process is attached to the second, instead of the third, setiger; the latter, however, remains a possibility (18). No setae other than very long fine larval capillaries can be detected. We have been unable to find any previous record of a larval Cossura.

Chloraemidae

Flabelligera affinis Sars. Fauvel (13)

Friday Harbour, Wash., U.S.A., swarming at dock

Five specimens, the longest of which measures 20 mm, were taken at a night light by Dr. R. L. Fernald of the Friday Harbour Laboratories. They were sexually mature and spawning. The only reference we have been able to trace to the swarming of this species is given by McIntosh (28) as having been observed by Dr. H. C. Sorby in the River Orwell, in England. Its occurrence at Friday Harbour (which falls within the region dealt with in this paper) seems noteworthy.

There is no previous record of the occurrence of the species at Friday Harbour nor elsewhere on the northwest coast of North America, *F. infundibuliformis* (Johnson) being the only representative of the genus known hitherto in the region. The present specimens differ from *F. infundibuliformis*, and

agree more closely with *F. affinis*, in respect of the much smaller number of segments, less sharply curved hooks, and the possession of two types of stalked cuticular papillae. The two species are, however, very closely allied. Pettibone (29) regards them as synonymous.

Miscellaneous Larvae

In addition to the above many larvae too small or imperfect to identify to species occur in the material. Amongst these, Spionidae are the most frequent, many of them sufficiently advanced to place in the genus *Polydora* by the presence of the modified fifth setiger. Eunicidae (Dorvillea?) and several kinds of Polynoidae also occur. The distribution seems to be indiscriminate.

Poeobiidae

Poeobius meseres Heath. Heath (17), Pickford (29)

53° 13′ N., 160° 53′ W., 400–200 m, several
53° 49′ N., 159° 32′ W., 300–200 m, several
54° 17′ N., 146° 37′ W., 400–300 m, several
51° 51′ N., 146° 37′ W., 400–300 m, several
51° 48′ N., 153° 20′ W., 400–300 m, several
54° 26′ N., 158° 07′ W., 400–300 m, several
48° 20′ N., 175° 30′ W., 300–200 m, several
48° 10′ N., 171° 21′ W., 200–100 m, several
52° 01′ N., 169° 44′ W., 400–300 m, several
49° 19′ N., 175° 30′ W., 200–100 m, several
51° 43′ N., 155° 00′ W., 150-m tow, several
53° 33′ N., 148° 00′ W., 150-m tow, several

The first observations of this species in our plankton material were made in 1956. It was not, however, reported in 1957 (6) since its identity had not then been determined. All, except the last two, of the above records date from 1956 when it occurred plentifully between long. 146° N. and long. 175° N. in the vicinity of 50° lat. The last two records date from 1958; since that time it has been found frequently in samples from 150 m or deeper. The species was originally recorded in 1930 from Monterey Bay, California (17), and subsequently in the same locality and elsewhere off the coast of California (16). At about the same time it was taken off SE. Alaska (30). Since then it has been collected by a number of expeditions exploring the north Pacific. McGowan (26) summarizes the localities (Fig. 3) and indicates a lack of data on distribution in the region of the area covered in the present paper. It has always been taken at moderate to deep levels. Most of the examples we have examined have been in the contracted condition figured by Heath (17, Plate 1, Fig. 1); only a few had the ends of the palps and tentacles emerging. Through the kindness of Dr. O. Hartman we have, however, been able to examine specimens collected off northern California by Dr. Martin Johnson which show the anterior region fully emerged and expanded as shown in her figure (16, Plate 1, Fig. 6). The length of our specimens varied from 6 to 17 mm.

The genus *Poeobius*, with type species *P. meseres*, was described from material collected in Monterey Bay, California, by Heath (17) and was regarded as a member of a new family Poeobiidae, to be included, provisionally, in the Echiuroidea. It was, however, recognized that it had certain affinities with the Polychaeta. Classification with the Echiuroidea was accepted by later workers until disputed by Fisher (14), who stressed the characters in which it differs from the Echiuroidea and agrees with the Polychaeta, but, in the absence of several characters hitherto regarded as essential to Polychaeta, found difficulty in including it in that group either. He suggested the setting up of a new phylum Poeobioidea to accommodate it. Pickford (30) whilst agreeing with Fisher as to its exclusion from Echiuroidea considered that, in the light of such affinities with Polychaeta as Heath and Fisher had pointed out and of others she was able to indicate, classification in that group was justified and concluded that it is an "aberrant polychaete". We are inclined to share Fisher's difficulty in including it in the Polychaeta in the complete absence of paired appendages, somatic segmentation, and setae.

Hartman (16) accepted Pickford's view and carried the argument in favor of polychaete analogy a stage further, suggesting its classification with the Flabelligeridae, but, in spite of the characters common to that family and *Poeobius*, which she indicated, the analogy is not altogether satisfying, in particular with regard to the comparison which is made between the gelatinous sheaths which occur in both. These differ fundamentally. That in *Poeobius* is internal, occupies a well-defined area between the hypodermis and the somatic musculature, and is sufficiently rigid to give the body in life considerable turgidity (17, p. 228). In *Flabelligera* the jelly is external, mucous, and follows only roughly the contour of the animal to form a protective covering.

On the whole there seems to be a case for classifying *Poeobius* amongst the Archiannelida, a group lending itself to the reception of such anomalous Annelida, until something is known of its development.

Siphonophora

Identification of the Siphonophora was kindly undertaken by Dr. G. O. Mackie, of the University of Alberta, who had already collected and identified some of the species recorded from localities in the region covered by the present paper. At his request these records are included here. They are indicated under each species by his initials or by those of the following collectors who sent him material: Mr. H. Menkes, of the Canadian Hydrographic Survey, Mr. A. Fontaine, of Victoria College, Victoria, B.C., and Dr. C. Carl, of the Provincial Museum, Victoria, B.C. Dr. Mackie wishes to acknowledge help he has received in the identifications from Mr. A. K. Totton of the British Museum.

Calcyophorae

Vogtia sp. Kölliker. Bigelow (9), Moser (25, Tafs. 17 and 18)
50° 00′ N., 145° 00′ W., 1200–0 m, one specimen, fragmentary

T. pentacantha Kölliker occurs in the Bering Sea and northwestern Pacific (9). It is characteristically a deep-sea form (25).

Dimophyes arctica Chun. Moser (25, Taf. 26)

50° 00′ N., 145° 00′ W., 150–0 m, one anterior nectophore

55° 58′ N., 150° 46′ W., 10 0 m, three anterior, one posterior nectophore (H. M.)

50° 00′ N., 145° 00′ W., 1200–0 m, one anterior nectophore

Collected in Bering Sea by U.S. Fisheries Steamer "Albatross" (9).

Muggiaea atlantica Cunningham. Moser (25, Taf. 1)

Ladysmith Harbour, V.I., 10-m tow, five complete polygastric stages and eight mature eudoxids (G.O.M.)

Friday Harbour, Wash., U.S.A. From float at night light, six polygastric specimens (G.O.M.)

A cosmopolitan species in little-contaminated coastal waters. Recorded from Monterey Bay, Cal., U.S.A. (10).

Physonectae

Agalma elegans Sars. Kawamura (19, unnumbered plate, Fig. 11), Totton (34, Fig. 24)

53° 30′ N., 138° 53′ W., 150–0 m, 29 adults, 16 larvae

52° 08′ N., 133° 26′ W., 20-m tow, one adult

48° 00′ N., 133° 12′ W., 150–0 m, seven larvae

53° 28′ N., 133° 32′ W., 150–0 m, five larvae

55° 00′ N., 150° 00′ W., 21–0 m, five larvae (H. M.)

55° 00′ N., 155° 00′ W., surface, five larvae (H. M.)

Occurs at Misaki, Japan (19). Not previously recorded from northeast Pacific.

Forskalia sp. Kölliker. Bedot (1), Totton (34, Figs. 29, 30, and 31)

54° 15′ N., 134° 40′ W., 100–0 m, one juvenile

This genus has not been recorded previously from the northeast Pacific.

Apolemia uvaria Lamarck. Moser (24, Fig. 507), Bigelow (8)

52° 02′ N., 136° 00′ W., 150–0 m, three specimens

This monotypic genus has not been recorded previously from the north Pacific.

Nanomia bijuga Delle Chiaje. Bigelow (8), Kawamura (19), Totton (34, Fig. 19D)

52° 00′ N., 127° W., 150–0 m, one nectophore

Oak Bay, Victoria, B.C., surface, two specimens (A. F.)

Friday Harbour, Wash., U.S.A. From below the wharf at night light. Twelve specimens, the largest with eight nectophores, the smallest with three. Luminescent, the luminescence appearing to correspond with the distribution of the reddish pigment (G.O.M.).

Occurs at Misaki, Japan (19). Not previously recorded from northeast Pacific.

Nanomia sp. (? *N. cara* Agassiz). Totton (34, Fig. 19A–C)
Oak Bay, Victoria, B.C., surface, two specimens (A. F.)
Between Sidney Island and Forest Island, B.C., one specimen (C. C.)
There is no previous record of *N. cara* from the north Pacific.

Mollusca

Prof. J. E. Morton, of the University of Auckland, New Zealand, was kind enough to classify the Mollusca in the collection and to him we extend cordial thanks. The quotations in the list which follows are from his notes. None of the species identified have been recorded previously from the northeastern Pacific.

HETEROPODA

Atlanta sp. Thiele (32)
51° 51′ N., 132° 00′ W., 150–0 m, one specimen
A second specimen, for which bearings are not available, "apparently of the same species" is in the collection. "The species is probably not the Pacific *fusca*, recorded at least as far north as San Francisco, but too small (only 1.5 mm) to fit this specimen. I should assign it to *A. gaudichaudi*, very widespread in the Pacific and relatively much more abundant there than the more dominant Atlantic species *A. peroni*."

PTEROPODA

Thecosomata

Euclio pyramidata Linné. Thiele (32, as *Clio*)
52° 18′ N., 134° 46′ W., 150–0 m, one specimen
50° 08′ N., 141° 02′ W., 150–0 m, one specimen

A third specimen, the bearings for which are not available, clearly belongs to this species. "The lateral processes are only faintly marked, but the lateral edges are characteristically distinct. The strong median dorsal rib is also clearly diagnostic." Two of the specimens are only sufficiently well preserved to be identified to genus with certainty, but "probably, on geographical grounds, the common species *pyramidata*".

Gymnosomata

Anopsia sp. Thiele (32)
53° 58′ N., 142° 22′ W., 150–0 m, one specimen
"Specimen in preserved state shell-less, and not easily determinable, though with a well marked toothed thecosome gizzard, upon dissection. With the transverse furrows marking the body, and the small glandular nodules in the loose skin, it has some of the characters of this problematic form."

Notobranchaea macdonaldi Pelseneer. Thiele (32)

52° 27′ N., 167° 21′ W., 150–0 m, one specimen

"Body shape much distorted and head and foot lobes alone give any clue as to the identity. Hook sacs, however, reveal the expected cluster of short wide hooks, and from what one can still detect there is a fair agreement with Notobranchaea. The Pacific distribution of this genus, however, is highly uncertain."

Thliptodon sp. (probably *diaphanus* Meisenheimer). Morton (23)

53° 23′ N., 133° 45′ W., 300–200 m, one specimen

Two further specimens, for which bearings are not available, are attributed to this genus. Characterized by "the general expansion of the head, the loosely fitting and very roomy "skin" surrounding the viscera and particularly the very roomy hook sacs, with a sheaf of rather golden-coloured long hooks". In one case "the everted hook sacs make it a fine specimen and give a good identification of character, with the golden chitinous appearance of the hook sheaves". "Of the Thliptodon spp. *diaphanus* is the best known of the Pacific ones, probably the only one, and occurs in the upper layers as well as the deeper water."

Cliopsis krohni Troschel. Massy (20), Thiele (32)

58° 29′ N., 140° 51′ W., 150–0 m, one specimen

Characterized by "the very small visceral nucleus inside a spacious gelatinous "skin" with small whitish glandular nodules, and a very long proboscis invaginated or coiled within. Also the very far forward position of the swimming wings".

Tunicata

Dr. Leo Berner, of the Scripps Institute of Oceanography, La Jolla, Cal., very kindly identified the following two species of pelagic Tunicata in material submitted to him. All were in the southern region of the area fairly close in shore.

DOLIOLIDA

Doliolum (*Dolioletta*) *gegenbauri* Ulianin. Berrill (7)

44° 00′ N., 127° 02′ W., 150–0 m

42° 05′ N., 127° 35′ W., 150–0 m

46° 08′ N., 127° 01′ W., 150–0 m

This species is widely distributed in the Atlantic, but, in the Pacific, its previously recorded distribution seems to have been confined to the more southerly region.

SALPIDA

Salpa fusiformis Cuvier. Berrill (7)

44° 00′ N., 127° 00′ W. (approximate), surface

42° 05′ N., 127° 35′ W., 150–0 m

Dixon Entrance, oblique tow, 100–0 m

This species is known from the Pacific coast of North America from California to Bering Sea (21).

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DIETARY MARINE FISH OILS AND CHOLESTEROL METABOLISM

2. THE EFFECT OF VITAMIN A AND LINGCOD LIVER OIL COMPONENTS ON THE SERUM CHOLESTEROL LEVELS IN CHICKS[1]

J. D. Wood

Abstract

Lingcod liver oil unsaponifiable material was separated into three main fractions by means of an aluminum oxide column. Major components of the three fractions were vitamin A, cholesterol, and glyceryl ethers, respectively. These fractions were given as dietary supplements to cholesterol-fed chicks and the effect of the supplements on the hypercholesterolemia induced by the cholesterol feeding was investigated. The fraction containing vitamin A prevented the hypercholesterolemia. Crystalline vitamin A acetate produced a similar effect when it was added as a dietary supplement. It was concluded that vitamin A was probably the hypocholesterolemic agent in lingcod liver oil although other compounds in the oil may also exert some influence on the control of the serum cholesterol concentrations in the chicks.

Introduction

Numerous reports have appeared in the literature in recent years on the effect of dietary fats and oils on cholesterol levels in the blood. These studies have included several which indicated that dietary fish oils can depress serum cholesterol levels in both man (1, 2, 3) and animals (4). The effectiveness of various marine fish oils in preventing hypercholesterolemia in cholesterol-fed chicks was studied by Wood and Biely (5), who found that lingcod liver oil and halibut liver oil were very efficient hypocholesterolemic agents. Further work in this laboratory (6) showed that the active factor in lingcod liver oil was present in the unsaponifiable fraction of the oil. This result was the opposite to that observed by De Groot and Reed (4) using rats. These workers found that cod liver oil depressed the serum cholesterol concentrations but that the depressant factor was present in the fatty acid fraction. The different effects observed with chicks and rats may be due to a species difference, or to the different composition of the fish oils employed in the investigations.

The liver oils of the lingcod and the halibut were most effective in preventing hypercholesterolemia in chicks as noted above. Moreover, these oils have a high content of vitamin A, being used commercially as a source of this vitamin. The possibility that vitamin A was the active factor in lingcod liver oil therefore appeared to be worth investigating. Experiments were carried out in an attempt to identify the hypocholesterolemic factor in lingcod liver oil unsaponifiable material and at the same time to study the influence of vitamin A on serum cholesterol levels in cholesterol-fed chicks.

Materials and Methods

Birds

New Hampshire chicks were used in the earlier experiments but more recently White Leghorn chicks were employed because they were more readily available.

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Lingcod liver oil was found to have similar effects on serum cholesterol levels in both types of birds. One-week-old cockerels were used in the experiments and they were standardized by weight to eliminate any possibility of variation in serum cholesterol levels due to differences in the size of the chicks.

Diets

The basal diet employed in the experiments was the same as that used previously (5). Cholesterol and the various dietary supplements were added in amounts detailed in the text and tables.

Determination of Serum Cholesterol

The birds were placed in groups of 16 and fed the appropriate diet and water ad libitum. After the required period of time the birds were bled from the wing and 0.5 ml blood collected from each chick. The blood from four birds was pooled and the serum collected in the normal manner. There were therefore four pooled samples of serum from birds on each of the diets. The free and total cholesterol contents of the samples were determined using the method of Sperry and Webb (7).

Preparation of Lingcod Liver Oil Unsaponifiable Material

One pound of lingcod liver oil was mixed thoroughly with 4 liters ethanol and 300 ml of a solution ($KOH:H_2O$, 3:2 w/v). The mixture was boiled gently under reflux for 30 minutes, during which time it was protected from light. The mixture was distilled until 2 liters ethanol had been removed and the remaining solution, after dilution with 4 liters water, was extracted several times with ethyl ether to obtain the unsaponifiable material. The above proportions of alcohol and water were chosen because they reduced to a minimum the formation of emulsions during the extractions. The ether extracts were combined and washed successively with water, dilute potassium hydroxide, and then with water until the washings were no longer alkaline to phenolphthalein. The solvent was removed on a water bath at 60° C under a stream of nitrogen and finally *in vacuo* at room temperature.

Preparation of Lingcod Liver Oil Fatty Acids

The saponification mixture, after removal of the unsaponifiable material, was acidified with hydrochloric acid and extracted several times with ethyl ether and the combined extracts washed with water. The solvent was removed as described above.

Fractionation of Lingcod Liver Oil Unsaponifiable Material

The fractionation was based on the method of Swain (8) and was carried out using a 75-mm diameter column containing 900 g of aluminum oxide (Woelm, basic, activity grade 1) equilibrated with 5% water. A slurry of the adsorbent in petroleum ether (b.p. 65–110°) was poured into the column which was fitted at the bottom with a perforated, fritted glass disk plus a thin layer of glass wool. The unsaponifiable material under investigation was dissolved in petroleum ether to give a final concentration of 2%. This solution was applied to the column followed by more petroleum ether, benzene, methylene chloride, and methanol in that order. The effluent was collected in fractions varying in

volume from 100 ml to 2 liters, the volume depending on the type and amount of material being eluted at that specific moment. The volumes of the fractions were chosen in the light of results obtained in previous runs and were smallest when the vitamin A and cholesterol were being eluted and largest towards the completion of the elution when nearly all the material had already been eluted.

In preliminary runs using aluminum oxide columns the benzene was omitted, the eluting solvent being changed directly from petroleum ether to methylene chloride. However, vitamin A and cholesterol were not separated very well by this method and it was found that the use of benzene as an intermediate solvent increased the efficiency of this separation. It was therefore used in the fractionation of the unsaponifiable material in the present investigation. The volumes of the individual solvents used are shown in Fig. 1.

Results

The chromatographic separation of the components of lingcod liver oil unsaponifiable matter is shown in Fig. 1. The material adsorbed on the aluminum oxide column was eluted in four major fractions. The first occurred with the benzene as solvent, the second and third with methylene chloride as solvent, and the fourth when the elution was carried out with methanol. The petroleum ether eluted only a trace of material. The recovery of the material from the column was excellent, 43.2 g of the original 43.6 g applied to the column being recovered in the eluate.

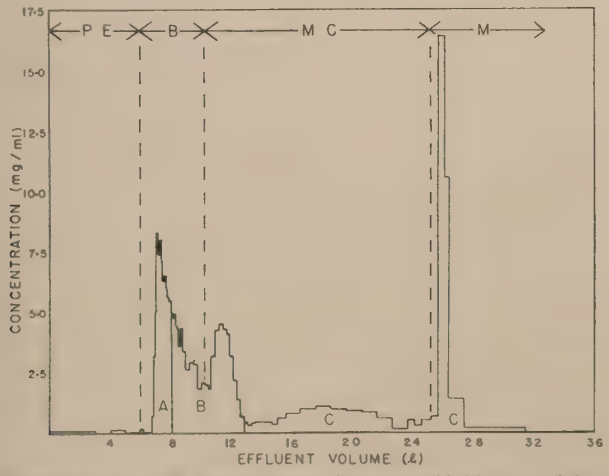

FIG. 1. Chromatography of lingcod liver oil unsaponifiable material on aluminum oxide. Solvents were petroleum ether b.p. 65°–110° (PE), benzene (B), methylene chloride (MC), and methanol (M).

The first peak in Fig. 1 contained large amounts of vitamin A which was identified by its ultraviolet absorption spectra and by the absorption spectra of its reaction product with antimony trichloride. The material under investigation showed maxima at 328 mμ and 620 mμ in these two tests, respectively. An accurate determination of the amount of vitamin A in the fractions was not

possible because of interfering substances, but the results obtained indicated that there was a considerable quantity of material present in the first peak which was neither vitamin A nor cholesterol. The semiquantitative estimation of vitamin A indicated that an appreciable amount of the vitamin had been destroyed during its isolation from the oil, possibly while the material was adsorbed on the column. The Liebermann–Burchard test for cholesterol indicated that the sterol was present in significant amounts in the fractions which made up the latter part of the first peak in the diagram. Vitamin A was also present in these fractions but not to the same extent as in the earlier fractions of the peak.

The second peak in Fig. 1 represents the material eluted immediately after the solvent was changed from benzene to methylene chloride. This material was solid and pale yellow in color and was almost entirely cholesterol as estimated by the Liebermann–Burchard reagent. This reagent is not specific for cholesterol and two further tests were carried out to identify the sterol. The time development of the color with the Liebermann–Burchard reagent was followed (9) and the development of the color was found to be identical with that obtained using a cholesterol standard. Further proof that the material was cholesterol was obtained from the melting point. The pale yellow solid melted at 141.5° C but recrystallization from 95% ethanol gave a white crystalline solid, m.p. 148° C. Further recrystallization had no effect on the melting point, which was identical with that observed with a sample of pure cholesterol. The mixed melting point of the two substances was also 148° C.

The compounds represented by the third and fourth peaks in Fig. 1 contained no traces of either vitamin A or cholesterol and their physical states were an oil and a waxy solid respectively. The glyceryl ether content of the two fractions was obtained, using the method of Karnovsky and Rapson (10). The amount of glyceryl ethers was calculated (as selachyl alcohol) from the amount of formaldehyde produced during periodate oxidation. Formaldehyde was estimated using the dimedon reagent and the identity of the aldehyde was checked by taking the melting point of the dimedon precipitate. The value obtained was 189°, which agreed with the value for formaldehyde–dimedon compound observed by Reeves (11). The materials represented by peaks 3 and 4 were found to contain 59.7 and 96.5% glyceryl ethers respectively.

The components of the unsaponifiable material, which were thus separated, were tested for their effectiveness in preventing hypercholesterolemia in cholesterol-fed White Leghorn chicks. The material was divided into three fractions, A, B, and C, as shown in Fig. 1. The limits of fraction A were chosen so that it contained most of the vitamin A and none of the cholesterol. Fraction B contained the material represented by the second peak in Fig. 1, plus the latter fractions making up the first peak since these fractions contained substantial amounts of cholesterol in addition to some vitamin A. Fraction B therefore consisted mainly of cholesterol with a little vitamin A. The material represented by the third and fourth peaks was combined to form fraction C, which contained mainly glyceryl ethers but also some unidentified compound or compounds.

TABLE I

The effect of dietary vitamin A and lingcod liver oil components on the serum cholesterol levels in chicks

Addition to the basal diet	Food consumption (g/chick/day)		Average weight gain (g)		Serum cholesterol (mg/100 ml) Free		Serum cholesterol (mg/100 ml) Total		Cholesterol in free state (%)	
	7 days	13 days	7 days	13 days	7 days	13 days	7 days	13 days	7 days	13 days
None	17.3	19.5	43	91	38 ± 1	36 ± 2	137 ± 13	115 ± 8	28 ± 4	31 ± 4
1% cholesterol (Ch)	16.4	19.7	41	93	84 ± 17	101 ± 16	289 ± 37	336 ± 29	28 ± 3	31 ± 5
Ch + 10% lingcod liver oil	17.3	19.3	42	77	26 ± 4	31 ± 5	116 ± 11	91 ± 12	23 ± 4	34 ± 1
Ch + fraction A*	15.1	20.0	41	89	58 ± 6	61 ± 3	218 ± 25	197 ± 28	27 ± 1	31 ± 3
Ch + fraction B*	17.3	20.7	43	92	84 ± 24	79 ± 5	285 ± 37	248 ± 46	29 ± 4	32 ± 6
Ch + fraction C*	16.7	19.6	39	85	88 ± 17	126 ± 30	271 ± 59	429 ± 119	33 ± 1	30 ± 3
Ch + 580,000 I.U. vitamin A acetate/lb	16.4	20.4	39	87	42 ± 4	44 ± 5	146 ± 20	163 ± 12	30 ± 2	27 ± 2
Ch + 290,000 I.U. vitamin A acetate/lb	16.2	19.0	40	86	54 ± 8	56 ± 12	180 ± 21	190 ± 18	30 ± 7	30 ± 9
Ch + 145,000 I.U. vitamin A acetate/lb	18.4	20.6	42	88	44 ± 6	48 ± 6	159 ± 27	141 ± 15	28 ± 1	34 ± 1
Ch + 72,500 I.U. vitamin A acetate/lb	17.1	19.7	43	89	48 ± 5	62 ± 14	166 ± 24	175 ± 12	29 ± 5	35 ± 6
Ch + 36,250 I.U. vitamin A acetate/lb	18.1	19.9	41	83	58 ± 8	77 ± 12	217 ± 38	240 ± 36	27 ± 1	32 ± 1
Ch + 18,125 I.U. vitamin A acetate/lb	17.0	20.2	42	88	57 ± 6	65 ± 7	217 ± 24	196 ± 41	26 ± 1	33 ± 9
Ch + 9,062 I.U. vitamin A acetate/lb	16.7	21.1	43	90	53 ± 2	66 ± 19	201 ± 14	196 ± 39	27 ± 2	34 ± 4
Ch + 330,000 I.U. vitamin A alcohol/lb	18.0	20.7	45	89	52 ± 15	69 ± 14	195 ± 47	234 ± 45	26 ± 2	29 ± 2

*Fractions A, B, and C were added to 10 lb of the basal diet.

NOTE: Each cholesterol value in the table is the mean for four groups ± the standard deviation.

The effect of supplementing the diet of cholesterol-fed chicks with fractions A, B, and C and lingcod liver oil are shown in Table I. The food consumption of the chicks and their weight gain were similar for birds on all the diets after both 7 days and 13 days except for those on the lingcod liver oil supplemented diet. After 13 days on the latter diet the birds had consumed the normal amount of food but their weight gain was somewhat less than that of birds on the other diets.

The influence of the different dietary supplements on the prevention of hypercholesterolemia in the chicks was varied. After 7 days the chicks fed diets supplemented with fraction A or lingcod liver oil exhibited serum cholesterol levels which were lower than the levels in the cholesterol-fed control birds. Analysis of variance indicated that the differences were significant at $P = .01$. The same level of significance is used in all cases in this paper where a significant difference is mentioned. Fraction A and lingcod liver oil produced similar effects after 14 days on the diet. Fraction B had no effect on the serum cholesterol levels in cholesterol-fed chicks after 7 days on the diet but at the end of 13 days a significant lowering of the cholesterol concentration was observed. Fraction C produced no effect after 7 days but after 13 days on the diet the cholesterol levels were significantly higher than the cholesterol-fed controls. A group of chicks was placed on the basal diet without any added cholesterol and it was observed that lingcod liver oil lowered the serum cholesterol concentration in cholesterol-fed chicks to a value below that in the chicks on the basal diet. The other fractions capable of reducing the rise in serum cholesterol did not produce such a strikingly low level of cholesterol.

The alterations in the serum cholesterol levels brought about by the dietary supplements applied both to the free cholesterol and to the total amount of the compound. Dietary supplements which drastically changed the levels of serum total cholesterol had no effect on the percentage of cholesterol in the free state. The results in Table I show that this percentage composition varied little, not only between birds on the same diet, but also between birds on different diets.

The ability of vitamin A to prevent hypercholesterolemia in cholesterol-fed chicks is shown in Table I. Both the alcohol and acetate form of the vitamin significantly lowered the serum cholesterol concentration. Although the hypocholesterolemic effect of the vitamin A acetate increased somewhat with concentration, the increase was rather erratic and it appeared that vitamin A concentration was not too critical at concentrations greater than 9062 I.U./lb feed. The values of vitamin A shown in Table I indicate the amount of the crystalline vitamin added to the diets. All diets, in addition to this supplement, contained 2500 I.U./lb vitamin A derived from feeding oil used to formulate the basal diet. None of the vitamin A supplemented diets lowered the serum cholesterol levels to the same extent as did the lingcod liver oil supplemented diet. The fish oil was rich in vitamin A (42,500 I.U./g), which would result in an addition of 1,929,500 I.U. of vitamin A per pound of feed. This level is approximately three times greater than the highest level of crystalline vitamin A added to the diets. The action of the dietary vitamin A was similar to that of the fish oil and its components in that it had no effect on the proportions of cholesterol

in the free and esterified states, respectively. The amounts of the basal diet and vitamin A supplemented diets consumed by the chicks were similar. In this respect chicks differ from rats, which were found to eat less of the vitamin A supplemented diets (unpublished results).

As mentioned previously, the unsaponifiable portion of lingcod liver oil contained the hypocholesterolemic factor whereas the fatty acid portion obtained after the saponification of the oil increased the degree of hypercholesterolemia in cholesterol-fed chicks (6). The effect of the unsaponifiable material on the hypercholesterolemia caused by the fatty acids was therefore studied because the results of such an experiment might shed some light on the mechanism involved in the control of serum cholesterol levels by dietary means.

Lingcod liver oil was saponified and the unsaponifiable material and free fatty acid fractions were isolated as described above. The whole oil and the two fractions (singly and combined) were added to the diets of New Hampshire chicks as shown in Table II. The amount of unsaponifiable material which

TABLE II

The effect of dietary lingcod liver oil and its components on the serum cholesterol level in chicks

Addition to the basal diet	Serum cholesterol (mg/100 ml), 10 days
None	119 ± 5
1% cholesterol	258 ± 51
1% cholesterol + 10% oil	83 ± 9
1% cholesterol + 300 g fatty acid/10 lb feed	315 ± 58
1% cholesterol + unsaponifiable material	110 ± 18
1% cholesterol + 300 g fatty acids + unsaponifiable material	137 ± 16

NOTE: Each value in the table is the mean for four groups ± the standard deviation.

was used to supplement the diets was chosen so that it was equivalent to that obtained from the quantity of oil used as the dietary supplement. In agreement with previous results the oil and unsaponifiable material prevented the induced hypercholesterolemia and the free fatty acids enhanced it. The unsaponifiable material was able to counteract the effect of the fatty acids when the two fractions were both added to the diet. The whole oil and the unsaponifiable portion not only prevented the induced hypercholesterolemia but in addition they produced serum cholesterol levels which were lower than those of the chicks on the basal diet.

Discussion

The results observed when the components of lingcod liver oil unsaponifiable material were added as dietary supplements to cholesterol-fed chicks would indicate that vitamin A may be the hypocholesterolemic factor in the fish oil. Of the fractions tested, that containing the main portion of vitamin A was the most effective in preventing the rise in serum cholesterol induced by dietary cholesterol. The fraction containing a small amount of the vitamin produced a significant effect only after 13 days on the diets, there being no significant

prevention of the hypercholesterolemia after 7 days. The fraction which was devoid of vitamin A increased the cholesterol-induced hypercholesterolemia. The activity of the isolated vitamin A was not directly comparable with that of the oil because a certain amount of the vitamin had been destroyed during the isolation and the remainder was not contained wholly in any one fraction.

The results obtained with crystalline vitamin A acetate would appear to substantiate the hypothesis that vitamin A is the active factor in lingcod liver oil. The crystalline vitamin at all levels investigated, 9062 I.U./lb feed to 580,000 I.U./lb feed, curtailed the rise in serum cholesterol due to dietary cholesterol.

However, the effect of crystalline vitamin A acetate was not so great as that of the lingcod liver oil, nor for that matter the unsaponifiable fraction of the oil. The latter two substances when added to a cholesterol-containing diet produced serum cholesterol levels which were lower than those found in the chicks on the basal diet without added cholesterol. The greater potency of the fish oil may be due to its high content of vitamin A. The amount of the vitamin added to the diet in the oil was three times as much as the highest concentration of crystalline vitamin A tested. However, the rather indefinite increase in the hypocholesterolemic effect with increase in the vitamin A concentration would seem to make this improbable. Moreover, lingcod liver oil with a lower vitamin A content was used in a previous experiment (5). In this case the oil-supplemented diet contained 900,000 I.U./lb feed, which is of the same order of concentration as the 580,000 I.U./lb feed used here, and yet the oil prevented completely the induced hypercholesterolemia as compared with the partial prevention effected by the crystalline vitamin. The possibility therefore exists that there is some compound or compounds apart from vitamin A which are present in lingcod liver oil and which play some role in the regulation of serum cholesterol levels. This unknown substance may itself have hypocholesterolemic properties or it may stimulate the activity of the vitamin A. Take, for example, the glyceryl ethers which are present in appreciable quantities in lingcod liver oil. They produce no lowering of serum cholesterol concentrations per se but they are known to act as emulsifying agents. The potent hypocholesterolemic activity of fish liver oil may be due to the ability of the glyceryl ethers to increase the degree of dispersion of vitamin A in aqueous media. These suggestions are of course speculative and studies are now being planned to investigate this aspect more thoroughly.

The effect of vitamin A on blood cholesterol levels in poultry has been studied previously by Weitzel *et al.* (12). These workers gave large oral doses of vitamin A to old atherosclerotic hens, and although a marked anti-atherosclerotic effect was observed, the changes in the serum cholesterol were slight. These results are contrary to the present findings but Weitzel and his co-workers were studying the lowering of cholesterol levels as distinct from the present study, which was devoted to the prevention of a rise in serum cholesterol concentration due to dietary cholesterol. In addition, the cholesterol metabolism may be quite different in young cockerels and old hens. The two investigations are not therefore comparable.

The present investigations give little or no indication as to the site of the hypocholesterolemic activity of vitamin A. Petersen (13) prevented the onset of hypercholesterolemia in cholesterol-fed chickens using dietary β-sitosterol, and the mechanism of this action has been investigated. It is now postulated that the inhibition occurs due to interference with the esterification of cholesterol which occurs during its transfer from the intestinal lumen to the lymph, probably at the site of transfer to the central lacteal (14). The possibility that vitamin A acts at the same point cannot be excluded but if the mode of action is similar then vitamin A is much the more potent in its action. Petersen *et al.* 15) showed that a ratio of 2:1 between β-sitosterol and cholesterol prevented completely the hypercholesterolemia due to the cholesterol. They found that the action of β-sitosterol decreased rapidly with lowering in concentration and that a ratio to cholesterol of 1:4 was only 50% effective. The same degree of protection was afforded by a vitamin A to cholesterol ratio of 1:1500. The fact that free fatty acids in the diet increased the hypercholesterolemia and that this effect was counteracted by the lingcod liver oil unsaponifiable material suggests that the esterification of the cholesterol may possibly be the site of action of the hypocholesterolemic material.

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A NEW NEMATODE OF THE GENUS CUCULLANUS (CAMALLANATA : CUCULLANIDAE) FROM A FLOUNDER, PAROPHRYS VETULUS GIRARD, 1854, WITH NOTES ON THE SPECIES FROM PLEURONECTIFORMES[1]

L. Margolis

Abstract

Cucullanus annulatus n. sp., parasitic in the intestine of the pleuronectiform fish *Parophrys vetulus* Girard from coastal waters of British Columbia, is described. The species of *Cucullanus* known from Pleuronectiformes are reviewed. *Cucullanus wülkeri* Kreis is considered a synonym of *C. heterochrous* (Rudolphi).

The lemon sole or English sole, *Parophrys vetulus* Girard, 1854, a member of the family Pleuronectidae of the order Pleuronectiformes (= Heterosomata), is found along the Pacific coast of North America, from southern California to northwestern Alaska. In the coastal waters of British Columbia this fish harbors a species of *Cucullanus* which does not appear to have been reported previously. The name *Cucullanus annulatus* n. sp. is proposed for it.

The material examined consists of the following: 14 males collected from one host from Baynes Sound, east coast of Vancouver Island, on October 12, 1951; 5 males, and 11 males and 8 females, collected from two hosts, respectively, from near Dodd's Narrows, east coast of Vancouver Island, on December 30, 1952; 5 males and 8 females collected from one host from Union Bay, east coast of Vancouver Island on May 25, 1954; 14 males and 5 females, and 9 males and 7 females, collected from two hosts, respectively, from near Dodd's Narrows on March 28, 1958; and 26 males and 15 females collected from one host from near Dodd's Narrows on May 4, 1959.

Measurements in the following description are based on 41 males and 19 mature (egg-bearing) females. Unless otherwise stated, measurements in the description of the female refer only to the mature specimens. No attempt was made to separate the males on the basis of maturity or immaturity.

Description of *Cucullanus annulatus* n. sp.

(Figs. 1–13)

Males are small, slender nematodes, 2.21 to 5.50 mm in length, with the anterior end bent dorsad to a varying degree and the tail curved strongly ventrad. The slightly swollen head measures 0.12 to 0.18 mm in diameter.

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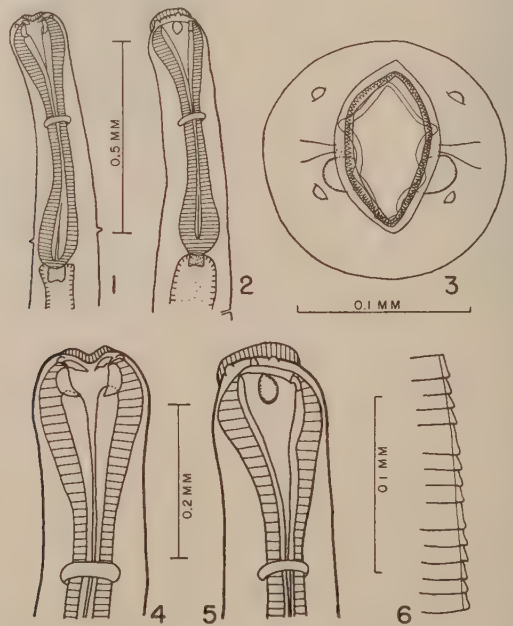
Behind the head the body narrows towards the midoesophageal region, then enlarges again to a width of 0.10 to 0.25 mm just behind the oesophagus. In specimens longer than about 3.5 mm the maximum body diameter is attained at this level. In specimens up to about 3.5 mm in length, this postoesophageal body diameter is usually slightly less than the diameter at the head end. The body tapers more or less gradually from the postoesophageal region to the cloacal opening and then abruptly to the pointed posterior extremity. The tail is 0.083 to 0.13 mm in length.

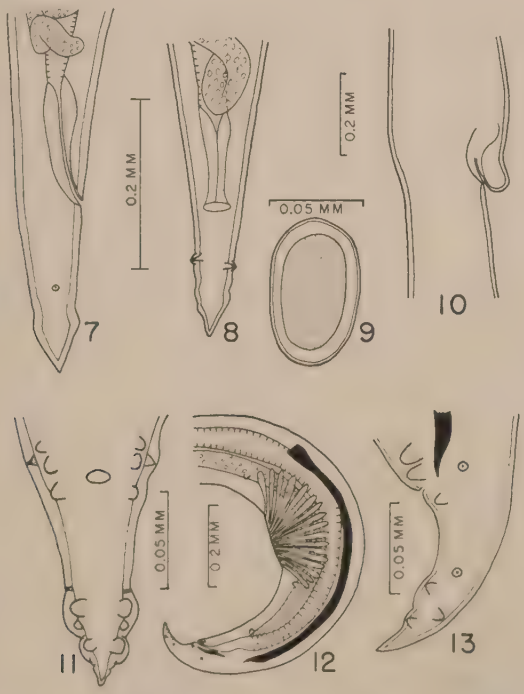
The cuticle is 0.002 to 0.005 mm thick. It appears to be devoid of transverse striations in the posterior half of the body. At about mid-length of the body, very fine cuticular striations are barely discernible. Proceeding anteriorly the striations gradually become more definite and farther apart. They are more distinct ventrally than dorsally. Ventrally, from a short distance posterior to the oesophageal-intestinal junction to the anterior oesophageal swelling, the striations are so prominent as to be more properly termed annulations. These annulations become less marked laterally and are completely absent dorsally. When viewed laterally the cuticle of the anteroventral region has a serrated appearance as a result of the prominent annulations. The distance between the annulations gradually decreases anteriorly and is 0.0042 to 0.0097 mm.



FIGS. 1–6. *Cucullanus annulatus* n. sp. All figures drawn with the aid of a camera lucida.
FIGS. 1 and 4. Anterior end of male, ventral view.
FIGS. 2 and 5. Anterior end of male, lateral view.
FIG. 3. Anterior end of male, *en face* view.
FIG. 6. Cuticle of the ventral oesophageal region of female, lateral view.

The mouth is dorsoventrally elongated. Attached to the cuticular collar surrounding the mouth is a continuous membranous flange bearing many small teeth on its internal face. There are four submedian cephalic papillae and a pair of lateral amphids arranged as shown in Fig. 3. Internally there is a chitinous framework around the mouth opening. A pair of bean-shaped chitinous structures project posteriorly from a ventrolateral margin of the circumoral chitinous framework. In lateral view these structures are roughly oval-shaped and in ventral view they show a concave inner margin and a convex outer margin.

The oesophagus, 0.43 to 0.70 mm in length, is enlarged anteriorly, narrows towards its mid-length, and expands again to a club-shaped swelling posteriorly. The anterior swelling, 0.10 to 0.16 mm in diameter, is always more pronounced than the posterior one, which measures 0.065 to 0.11 mm in diameter. At its mid-length the oesophagus has a diameter of 0.038 to 0.057 mm. The ratio of oesophagus length to body length decreases from about 1:5 in the smallest specimen to about 1:8 in the largest specimen. The oesophagus



FIGS. 7–13. *Cucullanus annulatus* n. sp. All figures drawn with the aid of a camera lucida.
FIG. 7. Posterior end of female, lateral view.
FIG. 8. Posterior end of female, ventral view.
FIG. 9. Unsegmented ovum.
FIG. 10. Vulvar region, lateral view.
FIG. 11. Posterior end of male, ventral view.
FIGS. 12 and 13. Posterior end of male, lateral view.

is lined with chitin except for a short distance at the posterior end. A two-lobed valve projects from the oesophagus into the intestine. The intestine opens into a short rectum, which also receives the ejaculatory duct to form a cloaca. The cloacal opening may or may not be set on a prominent elevation, presumably depending on the state of contraction of the muscles in the caudal region.

The nerve ring is 0.21 to 0.30 mm from the anterior extremity. The lateral cervical papillae are situated 0.026 to 0.097 mm in front of the junction of the oesophagus and intestine or 0.37 to 0.59 mm from the anterior end. The left cervical papilla is often a little more anterior than the right one.

The excretory pore lies 0.057 to 0.17 mm behind the oesophagus.

The testis originates at about the level of or slightly anterior to the anterior margin of the preanal sucker. It follows a straight course anteriorly, reaching to within 0.08 to 0.60 mm of the oesophagus before turning on itself and proceeding backwards to join the seminal vesicle.

The preanal sucker, in lateral view, appears as a fan-shaped group of muscles which, when contracted, takes on the form of a cup-shaped sucker. In ventral view this sucker is oval-shaped. Its length is 0.14 to 0.29 mm and its posterior margin is 0.18 to 0.37 mm in front of the anus.

There are 11 pairs of caudal papillae. The two anterior pairs are the largest, one pair lying immediately anterior to the preanal sucker and the second pair lying just posterior to or on the posterior margin of the sucker. The third pair lies at about 2/5 to 3/5 the distance from the sucker to the anus. These three pairs of preanal papillae are subventral. The fourth and fifth pairs are also subventral, lying one behind the other immediately in front of and lateral to the anus. The sixth pair is lateral and is at about the same level as the fifth pair. The seventh pair, subventral, lies immediately behind the anus or at its posterior margin. The remaining four pairs of papillae lie some distance behind the adanal group. The eighth pair is lateral and is the smallest of all the caudal papillae. The most posterior or 11th pair is subventral. Of the two pairs of papillae between the 8th and 11th pairs, one pair is lateral or subdorsal and the other pair is subventral. The relative position of these two pairs is somewhat variable. Usually the subventral papillae lie in front of or at about the same level as the lateral or subdorsal ones but occasionally they are situated behind the lateral or subdorsal pair.

The slender spicules, 0.47 to 0.76 mm in length, are pointed distally and slightly enlarged proximally. When completely retracted they extend to almost the middle of the preanal sucker or beyond. The accessory piece measures 0.065 to 0.085 mm in length by 0.0081 to 0.0097 mm in breadth.

Females are larger than the males. Mature (egg-bearing) specimens are 5.16 to 9.43 mm in length. Of two specimens of length 5.16 mm, eggs were present in the uterus of one but not in the other. The dorsal flexure of the head usually is not as pronounced as in the male. The body diameters at the head and at the posterior end of the oesophagus are 0.12 to 0.15 mm and 0.15 to 0.21 mm, respectively. The maximum body width, 0.17 to 0.27 mm, is

attained at about 1/6 the length from the anterior end. The posterior end tapers sharply from a short distance anterior to the anus to the pointed tip of the tail, which is 0.15 to 0.24 mm in length.

Nineteen immature females, excluding the specimen of length 5.16 mm, measured 1.94 to 4.68 mm in length. In immature specimens of length greater than 3 mm the body diameter increases to a maximum at the level of the junction of the oesophagus and the intestine and then decreases uniformly to the tail. In specimens less than about 3 mm in length the body tapers gradually from the head to the tail.

The cuticle is 0.004 to 0.008 mm thick. Faint cuticular striations were seen in the posterior half of the body of some and may be present in all specimens. At about mid-length of the body, fine striations are apparent in all specimens and proceeding anteriorly they become more distinct and farther apart. Approaching the junction of the anterior and middle thirds of the body the striations gradually transform into marked annulations which can be seen both dorsally and ventrally. Ventrally these annulations extend to the region of the anterior oesophageal swelling and dorsally they are present to the mid-length of the oesophagus. The distance between the annulations decreases anteriorly and is 0.0065 to 0.023 mm. In immature females the annulations are most prominent ventrally in the oesophageal region, similar to the condition observed in males.

The mouth and associated structures are as in the male.

The oesophagus has the same shape as in the male but the anterior swelling is less pronounced. The diameters at the anterior swelling, at the mid-length, and at the posterior swelling are 0.10 to 0.13 mm, 0.049 to 0.070 mm, and 0.087 to 0.12 mm, respectively. Generally, the diameter of the anterior oesophageal swelling is greater than that of the posterior swelling, the difference between these diameters decreasing with increased body length. In a specimen of 9.43 mm in length the posterior swelling is just slightly larger than the anterior one. In immature specimens the difference in diameters of the two oesophageal swellings is more pronounced than in mature specimens and reaches a maximum in the smallest specimen. The length of the oesophagus is 0.64 to 0.92 mm or 1/8 to 2/25 of the length of the body of mature specimens. Generally, the ratio of oesophagus length to body length increases with decreased body length and in an immature female of length 1.94 mm it is 1:4.3.

The nerve ring lies 0.28 to 0.33 mm from the anterior extremity.

The lateral cervical papillae are 0.07 to 0.16 mm anterior to the posterior end of the oesophagus or 0.53 to 0.76 mm from the anterior end of the body. Another pair of lateral papillae occur at about mid-length of the tail or a little farther behind.

The excretory pore is 0.10 to 0.29 mm posterior to the oesophagus.

The vulva, overhung by a very prominent anterior lip, is situated 3.1 to 6.1 mm (55.5% to 66.7% of total body length) from the anterior end. The vagina is directed anteriorly and joins the two divergent uteri, which, in fully mature individuals, contain many eggs. The eggs have a thin shell and measure 72 to

85 μ in length by 42 to 50 μ in width. The posterior ovary is 0.073 to 0.17 mm from the anus and the anterior ovary is 0.11 to 0.66 mm from the posterior end of the oesophagus.

Type host: Parophrys vetulus Girard (family Pleuronectidae).

Type locality: Dodd's Narrows, east coast of Vancouver Island, British Columbia.

Type specimens: Holotype male, allotype female, and paratypes to be deposited in the National Museum, Ottawa, Canada.

Discussion

The genus *Cucullanus* is a large one, more than 70 species having been described. Campana-Rouget (2), in a revision of the subfamily Cucullaninae, reported 65 species (plus 2 varieties), 16 of which are cited as *sp. inquirenda*. At least six more species are known, these having been described by Karve (8), Yamaguti (19), and Khera (9). The principal hosts are fishes. Only four species are known from other hosts, namely chelonians.

Cucullanus annulatus appears to be unique among this large group of species because the fine transverse cuticular striations become more marked anteriorly, giving rise to prominent annulations. These annulations impart to the margins of the cuticle a serrated appearance. Other species apparently do not show this transition from fine cuticular striations to annulations in the anterior region of the body. In three species from chelonians, namely *C. serratus* (Lane, 1916), *C. niloticus* Campana-Rouget, 1957, and *C. hardellus* Khera, 1956, prominent cuticular annulations (or serrations) are present in addition to the fine striations for almost the entire length of the body; the annulations do not replace the striations as in *C. annulatus*. These species from chelonians differ from *C. annulatus* in many other morphological characters, including the arrangement of the postanal papillae, absence of a very prominent anterior vulvar lip, position of the excretory pore, body and spicule lengths, and various other measurements.

Dacnitis squali Dujardin, 1845, from *Galeus galeus*, possesses a cuticle which seems to be most similar to that of *C. annulatus*. Dujardin (6) described the cuticle as follows: "tégument à stries transverses de Omm, 014, et paraissant denté en scie sur les bords". The brief description of one female does not permit a decision, on morphological grounds, as to whether the species is truly a *Dacnitis* or whether it should be transferred to *Cucullanus*. Even if it proves to be a species of *Cucullanus*, its large size (length of the female, 18.5 mm) would seem to exclude the possibility that it is identical with *C. annulatus*.

Up to the present time only one species, *Cucullanus smedleyi* Campana-Rouget, 1957 (= *C. elongatus* Smedley, 1933), has been recorded from marine fishes from the Pacific coast of North America. It was described by Smedley (14) from *Ophiodon elongatus* taken in British Columbia waters. *Cucullanus smedleyi* is a much larger species than *C. annulatus*; the males measure up to 30 mm in length and the females up to 40 mm in length. In addition to the extreme difference in size, with accompanying differences in measurements of

various structures, four features are noticeably different in the two species. (1) The cuticle of *C. smedleyi* lacks the annulations noted in the anterior region of *C. annulatus*. (2) The anterior region of *C. smedleyi* is not curved dorsally as is that of *C. annulatus*. (3) The oesophagus of *C. smedleyi* is divided into two regions, a coarsely muscular anterior portion (about 1/4 to 1/3 of the total oesophagus length) and a more finely muscular posterior portion. A similar distinction of two oesophageal regions is not apparent in *C. annulatus*. (4) The testis of *C. smedleyi* is much convoluted, whereas that in *C. annulatus* follows a straight course anteriorly and then posteriorly.

While differing considerably from *C. smedleyi*, *C. annulatus* shows strong similarities to several species of *Cucullanus*, particularly *C. heterochrous*, recorded from Pleuronectiformes from distant geographical regions.

Campana-Rouget (2) listed seven species of *Cucullanus*, one of which was not named, from Pleuronectiformes. These are *C. heterochrous* Rudolphi, 1802; *C. minutus* Rudolphi, 1819; *C. antipodeus* Baylis, 1932; *C. cylindricus* (Chandler, 1935) Campana-Rouget, 1957; *C. pleuronectidis* (Yamaguti, 1935) Campana-Rouget, 1957; *C. gendrei* Campana-Rouget, 1957; and *Cucullanus* sp. Campana-Rouget and Chabaud, 1956.

On the basis of available descriptions and illustrations, *Cucullanus annulatus* appears to differ consistently from these species not only in the possession of transverse cuticular annulations in the anterior region of the body but also in the possession of a very prominent anterior vulvar lip.

Notes on the Species of *Cucullanus* from Pleuronectiformes and Comparisons with *C. annulatus*

1. *Cucullanus heterochrous* is a well-known parasite of several species of Pleuronectidae and Soleidae from European waters and it has also been reported from the coast of French West Africa.

The measurements generally quoted for this species suggest that it is larger than *C. annulatus*. Törnquist (15), in a very detailed redescription of *C. heterochrous*, gave the following range of measurements for 10 specimens of each sex: males, 7.067 to 8.938 mm in length by 0.219 to 0.289 mm in maximum width; females, 8.674 to 11.186 mm in length by 0.281 to 0.406 mm in maximum width. In a footnote on page 74 he stated that he had not included in this range of measurements a male 9.158 mm and a female 12.543 mm in length. Also, in context, Törnquist gave spicule and other measurements of a male, 6.5 mm in length. The lengths of several apparently immature specimens were given by Törnquist as follows: the smallest individual was 1.763 mm in length; a female in which the vulva was poorly differentiated was 1.81 mm in length; a male in which the preanal sucker was weakly developed was 3.214 mm in length; and a male in which the preanal sucker was well marked was 4.321 mm in length. Törnquist's data were only partially utilized in recent publications by Dollfus (5) and Campana-Rouget (2), who cited the lengths of males and females as 7 to 9 mm and 8.6 to 12.5 mm, and 7 to 8.9 mm and 8.6 to 11.18 mm, respectively. In a paper preceding that of Törnquist, Gendre (7) recorded

the measurements of a male and female as 9.03 mm in length by 0.35 mm in width and 10.74 mm in length by 0.35 mm in width, respectively. More recently Kreis (10) reported the measurements of a male as 8.26 mm in length by 0.405 mm in maximum width, and those of females as 8.69 to 12.03 mm in length by 0.426 to 0.495 mm in width.

Smaller specimens, apparently mature, than those reported by the preceding authors have been described. Wülker (16) examined a large amount of material from *Pleuronectes flesus* and *P. platessa* from the North Sea and gave the size of males as 4.4 to 6.84 mm (mean 5.1 mm) in length by 0.20 to 0.23 mm (mean 0.23 mm) in width, and that of females as 6.3 to 10.4 mm (mean 8.0 mm) in length by 0.25 to 0.39 mm (mean 0.34 mm) in width. Punt (13) recorded measurements of one male and five females from *Pleuronectes* spp. from the North Sea as 5.9 mm in length by 0.2 mm in width, and 6.9 to 8.5 mm in length by 0.16 to 0.25 mm in width, respectively.

The measurements given by Wülker and Punt, both of whom recorded their specimens as *C. platessae* Rudolphi, 1809 (an accepted synonym of *C. heterochrous*), actually indicate that the size range of *C. heterochrous* overlaps with that of *C. annulatus*. Nevertheless, the measurements of body size given by various authors demonstrate that the maximum length of *C. heterochrous* is in excess of the known maximum length of *C. annulatus*.

Little difference exists between measurements of structures or organs of *C. heterochrous* and *C. annulatus* and such differences as do exist are approximately in proportion to the larger body size of *C. heterochrous*. Punt's measurement of 4.8 mm for the length of the spicules in a male of length 5.9 mm is probably a misprint for 0.8 or 0.84 mm.

Kreis (10) named *Cucullanus wülkeri* for Wülker's (16) specimens of *C. platessae* on the basis of the existence of only six pairs of caudal papillae in the male (three preanal and three postanal pairs) as described by Wülker. This small number of papillae in a species of *Cucullanus* is not in accord with the generic concept. The males of all species of *Cucullanus* are considered to be supplied with 11 pairs of caudal papillae. Wülker apparently overlooked the four pairs of adanal papillae and the smallest pair of postanal papillae. Törnquist (15, footnote, page 65) has already pointed out that Wülker's description of the caudal papillae was incorrect. It appears that *C. wülkeri* is another synonym of *C. heterochrous*.

2. *Cucullanus minutus* is another species common to some Pleuronectidae in Europe and has also received detailed morphological treatment by Törnquist (15). It is a shorter but considerably stouter worm than *C. annulatus* and possesses an intestinal caecum. According to Törnquist, males measure 2.62 to 3.39 mm in length by 0.27 to 0.39 mm in maximum width and females measure 2.53 to 3.49 mm in length by 0.34 to 0.53 mm in maximum width. Wülker (16) gave the size of the male and female as 3.7 mm by 0.39 mm and 5 mm by 0.56 mm, respectively. There are four pairs of subventral adanal papillae in males of *C. minutus* in contrast with three pairs of subventral and one pair of lateral adanal papillae in the males of *C. annulatus*, as well as in other *Cucullanus* spp. from Pleuronectiformes.

3. *Cucullanus antipodeus* was described by Baylis (1) from *Rhombosolea* sp. (family Pleuronectidae) from New Zealand. It is a larger species (males 6 to 8.7 mm in length and females 8.7 to 19.6 mm in length) than *C. annulatus*, but the spicules (0.45 to 0.5 mm in length) and gubernaculum (0.045 mm in length) are shorter. The cervical papillae, located a little behind the nerve ring, are farther forward than in *C. annulatus*, in which they are closer to the posterior end of the oesophagus than to the nerve ring. In *C. antipodeus* the eighth pair of caudal papillae in the male is lateral to the ninth pair and located at their base, whereas in *C. annulatus* the eighth pair of caudal papillae is lateral but distinctly anterior to the ninth pair. A unique feature of *C. antipodeus* is that the eggs contain coiled embryos at the time of deposition.

Khera (9) recorded a species as *C. antipodeus* from a non-pleuronectiform fish from Lucknow, India. He noted that his specimens differed from those of the original author of the species in the greater number of caudal papillae (14 pairs as compared to 11 pairs) and in a longer gubernaculum (0.077 mm as compared to 0.045 mm). The increased number of caudal papillae is due to a greater number of postanal rather than preanal papillae. Also, his illustration of the anterior end of a female shows the cervical papillae lying close to the posterior end of the oesophagus, whereas Baylis (1) described them as lying a little behind the nerve ring. In view of these differences, particularly in the number of caudal papillae, it is not unlikely that Khera was dealing with a species distinct from *C. antipodeus*. In fact the number of postanal papillae observed by Khera is unique among the Cucullanidae. In members of the genus *Neocucullanus* sensu Campana-Rouget, 1957 (2) there are more pairs of preanal papillae than in *Cucullanus*, but the number of adanal and postanal papillae is the same.

4. *Cucullanus cylindricus* was named by Chandler (4), as *Dychelyne cylindricus*, for *Ascaris* (?) sp. Linton, 1901 from *Paralichthys dentatus* (family Bothidae) from the Atlantic coast of the United States. This species is known only from females and is very poorly characterized. Nevertheless, from Linton's (11) brief description and illustrations, it is apparent that the species differs from *C. annulatus*. Linton stated that the diameter is about 1/10th of the body length, thereby being a much stouter worm than *C. annulatus*, and a long dorsal intestinal caecum as well as a short ventral intestinal caecum are present. *Cucullanus annulatus* lacks an intestinal caecum. Linton gave the body measurements of an apparently gravid female as 4 mm in length and 0.38 mm in maximum diameter, indicating that the species is not only stouter, but is also shorter than *C. annulatus*.

In a later paper, Linton (12) assigned *Ascaris* (?) sp. to *Heterakis* sp. and recorded a female specimen, which he stated "is similar to *Ascaris* (?) sp.", from *Lophopsetta maculata* (family Bothidae), and several females, which he stated "appear to be identical with *Ascaris* (?) sp.", from *Paralichthys albiguttus*. Campana-Rouget (2) considered these specimens to be cospecific with *C. cylindricus*. Accurate specific determination of Linton's material will require a re-examination of his specimens or a study of new collections from the same hosts and localities.

5. *Cucullanus pleuronectidis* was described by Yamaguti (17, 18), from various flatfishes of the families Pleuronectidae and Bothidae from Japan. Males measured 3.15 to 8 mm in length by 0.175 to 0.35 mm in width, and ovigerous females measured 5.5 to 11 mm in length by 0.3 to 0.65 mm in width. This species thus attains a greater length than that known for *C. annulatus* and is relatively somewhat stouter. The length of the gubernaculum, 0.033 to 0.04 mm, is about 1/2 of that in *C. annulatus* and the preanal sucker, 0.51 to 0.65 mm in front of the anus, is more anteriorly located than that in *C. annulatus*, in which the corresponding measurement is 0.18 to 0.37 mm. A ventral intestinal caecum, absent in *C. annulatus*, is present in *C. pleuronectidis*. The vagina in *C. pleuronectidis* is directed posteriorly rather than anteriorly as in *C. annulatus*. According to the illustration of *C. pleuronectidis* in Yamaguti's (17) earlier paper, the posterior oesophageal swelling is much more pronounced than the anterior one, but the measurements given in the later paper (Yamaguti (18)) suggest that in the smaller specimens the anterior swelling may be more marked. In *C. annulatus* the anterior oesophageal swelling is greater than the posterior one except in the largest female.

6. *Cucullanus gendrei* was described by Campana-Rouget (2) from *Syacium micrurum* (family Bothidae) from the coast of French West Africa. This species again is stouter than *C. annulatus* but the females are shorter. Measurements recorded for the males and females are, respectively, 3.03 to 4 mm in length by 0.31 mm in width and 3.2 to 6 mm in length by 0.38 mm in width. However, it was not stated whether all females in this length range were egg-bearing. Other differences in measurements between *C. gendrei* and *C. annulatus* are the longer tail, longer gubernaculum, longer oesophagus, greater distance of the nerve ring from the anterior end of the body, and the smaller eggs in the former species. The excretory pore lies anterior to the end of the oesophagus in *C. gendrei* but posterior thereto in *C. annulatus*. The posterior oesophageal swelling is more pronounced than the anterior one in *C. gendrei*, whereas the reverse prevails in *C. annulatus*, and a prominent postanal lip is present in *C. gendrei* but not in *C. annulatus*.

7. *Cucullanus* sp. Campana-Rouget and Chabaud, 1956 (3) is known only from three females taken from *Solea solea* from near Banyuls on the Mediterranean coast of France. Although they are about the same length as *C. annulatus* they are apparently a little stouter. A specimen of *Cucullanus* sp. of 8.23 mm in length had a maximum width of 0.35 mm, whereas females of *C. annulatus* up to 9.43 mm in length have a maximum width of 0.27 mm. The tail (0.27 mm) is a little longer and the eggs (0.061 to 0.067 mm by 0.038 mm) smaller in *Cucullanus* sp. The cervical papillae are at the level of the posterior extremity of the oesophagus in *Cucullanus* sp., whereas in *C. annulatus* they are located anterior to this region.

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MARINE STEROLS

VI. STEROL BIOSYNTHESIS IN MOLLUSCS AND ECHINODERMS[1]

U. H. M. Fagerlund and D. R. Idler

Abstract

The in vivo incorporation of 2-C^{14}-acetate into digitonin-precipitable material has been demonstrated in two molluscs, mussel (*Mytilus californianus*) and clam (*Saxidomus giganteus*). Clams are able to convert 11,14-C^{14}-squalene into digitonin-precipitable material. When the azoylester of the material isolated from clams is chromatographed, the major portion of the radioactivity follows the least polar zone, which has previously been found to contain mainly mono-unsaturated Δ^5-sterols.

A starfish (*Pisaster ochraceus*) has been shown in vivo to convert ingested 4-C^{14}-cholesterol to 7-cholestenol.

Introduction

Much information is available concerning the component sterols of marine invertebrates (1, 2, 3). Several phyla have yielded a large number of sterols, differing in the length of the side chain and the amount of unsaturation in the nucleus as well as in the side chain. A comparison of the component sterols of a particular species with those present in the food often reveals the absence of the ingested sterols. On the other hand, the sterols of a particular species seem to vary within certain limits with the locality and (or) the season (4). One must therefore assume either the presence of fairly active biochemical systems, which are able to convert exogenous sterols to those deposited by the animal, or the ability of the animal to dispose of the ingested sterols and to synthesize the sterols which it lays down in its body tissues.

A taxonomic classification of marine invertebrates, based on the type and number of sterols present, has been suggested. It appears that the evolutionary trend is towards cholesterol, which becomes the major sterol in the most advanced forms (1, 2, 5).

The present investigation was undertaken in order to establish (*a*) whether pelecypods are able to synthesize sterols from certain well-established precursors, and (*b*) whether starfish can transform the sterols in the food, essentially the Δ^5-type, to the Δ^7-type.

Part A

Biosynthesis of Sterols from Acetate and Squalene in Molluscs

The incorporation of acetate into squalene and the cyclization of squalene to cholesterol has been extensively studied in mammalian tissues (6). In insects there are indications that acetate is incorporated into the steroid side chain only (7). A beetle has been shown to be unable to synthesize sterols from acetate and other precursors (8), although sterols play both a metabolic and a structural role in these insects (9). Nymphs of German cockroaches (*Blattella germanica*)

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are able to convert exogenous sterols to those required by the organism (10). Sterol biosynthesis has been observed in some protozoa (11, 12). Some strains of yeast are able to synthesize ergosterol and zymosterol from acetate, squalene, and lanosterol but zymosterol is not a precursor of ergosterol (13, 14). Carbon 28 of ergosterol is not derived from 2-carbon units but rather from formate, serine, and methionine (15, 16).

In this investigation 2-C^{14}-acetate was injected into a mussel (*Mytilus californianus*) and a clam (*Saxidomus giganteus*) and a labelled sterol fraction was obtained. Although no direct proof has been obtained that squalene is an obligatory intermediate, its role as a precursor has been established by obtaining a radioactive sterol fraction from mussels injected with C^{14}-labelled squalene. Sterol synthesis in marine invertebrates from acetate or squalene has not been reported previously.

Methods

Radioactivity was determined in this part of the investigation with a counter equipped with a mica window Geiger–Mueller tube.

Injection of 2-C^{14}-Acetate into Molluscs

Mussels and clams were held in aerated aquaria supplied with running, filtered sea water and fed from time to time with macerated brine shrimp. After an animal had received an injection it was kept in a separate aquarium. A quantity of 2-C^{14}-acetate (specific activity (S.A.) 1 mc/23.6 mg) was dissolved in 0.05 ml of a 0.05 *M* phosphate buffer of pH 7.4, and the solution was injected at random into the body with a 22-gauge hypodermic needle forced in between the closed shells.

The oil fraction was obtained by macerating the soft part of the animal in a Waring Blendor or a Servall Omni-Mixer and extracting the mixture three times with hot acetone. After saponification with KOH and ether extraction in the usual manner the sterols from each animal were separated from the rest of the nonsaponifiable matter by precipitation with digitonin from 150 ml of water:acetone:ethanol 1:2:3. The sterols were separated into four zones as previously described by chromatography of the azoyl esters on silicic acid – Celite 535 columns using petroleum ether – benzene to develop the chromatograms (17).

Table I shows the distribution of radioactivity in the nonsaponifiable fraction and in the digitonides.

TABLE I

Radioactivity in nonsaponifiable matter and digitonides after injection with 2-C^{14}-acetate

Expt. No.	Animal used	Time between injection and extraction	Total radioactivity recovered in animals	Nonsap. in % of total C^{14} in animals	Digitonides in % of C^{14} in nonsap.
1	1 mussel	21 hours	4.6×10^5 c.p.m.	1.9	2.3
2	1 clam	2 to 10 days	8.3×10^6 c.p.m.	0.78	3.1
3	2 clams	11, 21 days	6.4×10^6 c.p.m.	0.62	4.5
4	1 clam	44 days	8.8×10^5 c.p.m.	0.47	8.4

In experiment No. 2 in Table I, radioactive material was injected in portions over a period of 8 days, each time in 0.1 ml of 0.1 *M* phosphate buffer. Two days after the last injection, the animal was extracted. Of the total activity in the flesh, 1.26×10^5 c.p.m., or 1.52%, about twice the activity in the nonsaponifiable material, remained in the oil fraction after it had been washed free of water-soluble activity.

Injection of C^{14}*-Squalene*

A clam was injected with 2.9 μc of 11,14-C^{14} squalene (specific activity 9.0 μc mg) of biological origin* suspended in 0.2 ml of a dilute Tween solution. The clam was extracted 18 days later as described above. Of the total activity recovered from the clams 1.6% was precipitated with digitonin. Cleavage of the digitonide converted the radioactive compound to an ether-soluble material (sterol).

Results

The experiments reported in this paper show that two species of Pelecypoda, mussel and clam, are able to utilize acetate for the synthesis of digitonin-precipitable material. In two experiments with clam this material was further investigated through chromatography of the azoylester. The major part (80%) of the radioactivity followed the least polar zone, which has previously been found to contain mainly monounsaturated Δ^5-sterols (17, 18). An insignificant amount of radioactivity was found in the zone containing 24-methylenecholesterol. In view of the fact that the clams under investigation, in accord with previous findings, contained more 24-methylenecholesterol than monounsaturated Δ^5-sterols, both being major components, it is of interest that the major portion of the activity resides in monounsaturated Δ^5-sterols.

It is not possible at this time to establish with certainty whether acetate is incorporated into sterols by way of condensation of two-carbon units or whether it enters into some specific part of the sterol molecule. However, the observation that labelled squalene is converted to sterols makes the first possibility the more likely of the two.

Part B
Conversion of Cholesterol to 7-Cholestenol in a Starfish

Studies of the sterol composition of oils from marine invertebrates conducted in this laboratory have revealed the presence of a doubly unsaturated sterol, 24-methylenecholesterol, in a number of pelecypods (17, 19). Recently the isolation of a closely related sterol, 7,24(28)-ergostadienol, from a starfish (*Pisaster ochraceus*) has been reported by us (20). Starfish are known to feed on pelecypods and hence ingest 24-methylenecholesterol. It therefore became reasonable to assume a biochemical relationship between these two 24-methylene sterols. Furthermore, since the sterols isolated from starfish (Asteroidea) to date have been C-7 (8) unsaturated, including 7-cholestenol (1, 2, 3), although the ingested sterols are mainly of the Δ^5-type, it appears that there

*Obtained from Commissariat à L'Energie Atomique, Seine-et-Oise, France.

may be present in starfish an efficient mechanism for the transformation of Δ^5-sterols to Δ^7-sterols. Through the use of tracer techniques we have now obtained evidence for the in vivo conversion of cholesterol to 7-cholestenol in starfish. This is the first report of a biological transformation of this kind. The reverse reaction, conversion of 7-cholestenol to cholesterol, has been observed in rabbit and in rat liver homogenates (21, 22). 7-Dehydrocholesterol is converted to cholesterol in rabbit (23) and the two sterols are interconvertible in guinea pig intestines (24).

Methods

Radioactivity was determined in this part of the investigation with a gas-flow counter equipped with a "micromil" window.

All animals were held in aerated aquaria supplied with running, filtered sea water. Cholesterol-4-C^{14} was dissolved in a few drops of salmon oil and the oil was injected into the body of a live mussel (*Mytilus californianus*). The mussel was then fed to a starfish that had been starved for a few days. The mussel was immediately consumed by the starfish. Nine days later the organs of the starfish were removed from the body cavity and macerated in a Waring Blendor. The sterol fraction was obtained as described above for clam sterols. On a preliminary chromatogram on silicic acid – Celite 535 (17) the sterol azoylesters separated into three zones. The bottom zone, in which unchanged cholesterol would be found, contained 436,000 c.p.m.; the middle zone in which 7-cholestenol would be found contained 150,600 c.p.m., and the top zone, in which 7,24(28)-ergostadienol is located (20), showed no significant amount of radioactivity. The middle zone was rechromatographed together with 30 mg of cholesteryl azoate. The activity remained in the upper zone. The upper zone on this chromatogram was cut to obtain only the middle, concentrated portion of the zone. This portion contained 90,400 c.p.m. It was crystallized once from benzene – aqueous methanol, hydrolyzed, and acetylated. To 13.4 mg of this material was added 120.6 mg of 7-cholestenol acetate prepared synthetically, m.p. 117°. The combined acetate, S.A. 156 c.p.m./mg was crystallized six times from aqueous ethanol. The specific activity remained constant after the third crystallization at 109 c.p.m./mg.

Removal of $\Delta^{5,7}$-Sterols

Ultraviolet analysis of the crystallized material showed the presence of less than 0.2% of $\Delta^{5,7}$-sterols. To remove this contamination, 9.1 mg of acetate and 5 mg of maleic anhydride were refluxed in *p*-xylene for 16 hours (17). After saponification with KOH the free sterol was crystallized three times from aqueous methanol. The S.A. of the material calculated as acetate was then 101 c.p.m./mg and it was free of $\Delta^{5,7}$-sterols.

Chromatographic Purification of 7-Cholestenyl Acetate

The six-times-crystallized acetate (190 μg, S.A. 109 c.p.m./mg) was applied as a short strip to Whatman No. 1 chromatographic paper impregnated with kerosene, b.p. 220–240° and the chromatogram was developed with 98% aqueous acetic acid for 64 hours at 25° C (25). The area corresponding to the concentrated portion of 7-cholestenyl acetate, applied as a standard, was cut

out and eluted. The 7-cholestenyl acetate content of the eluate was determined by the modified Liebermann-Burchard reaction. The S.A. was 113 c.p.m./mg.

Conversion of 7-Cholestenyl Acetate

Hydrogenation was carried out on 10 mg of the six-times-crystallized acetate with platinum oxide in glacial acetic acid. After two crystallizations the S.A. of the hydrogenation product, 8(14)-cholestenyl acetate, m.p. 85° C, was 122 c.p.m./mg.

Injection of 4-C^{14}-Cholesterol into Clams

A clam (*Saxidomus giganteus*) was injected with 4-C^{14}-cholesterol in a few drops of salmon oil and extracted 28 days later. The sterol azoylesters were obtained and chromatographed as described above. The middle zone contained 5.3% of the combined activity of all zones. The hydrolyzed and acetylated middle zone was combined with 17 parts of carrier 7-cholestenyl acetate to give a mixture of S.A. 155 c.p.m./mg. The material in three crystallizations had a S.A. of 65, 31, and 24 c.p.m./mg respectively.

Discussion

The method used in these experiments for introducing the radioactive material to the starfish was superior to a number of alternative methods that were tried. The loss of radioactive material was considerably greater when the salmon oil, containing 4-C^{14}-cholesterol, was injected with a blunt needle into the stomach or when a small gelatin capsule, containing the cholesterol–oil solution, was forced into the stomach through the mouth. An additional advantage of the adopted method was that the sterol entered the stomach of the starfish together with the food. The activity in the middle zone was 25.4% of the total in all zones. Of this activity 30% was lost on crystallization. The true activity of 7-cholestenol was therefore 17.8% of the total activity in the sterol fraction. There is evidence indicating that this is less than the maximal capacity of the starfish to carry out this transformation. In one experiment the activity in the middle zone was 82.5% of that in all zones on the chromatogram, but the result could not be reproduced in later experiments. The only apparent difference between this and subsequent experiments was that in this experiment the starfish received the radioactive material in two clams, the second clam 5 days after the first clam. In addition, it received a nonradioactive clam the second and third day after the first radioactive feeding. The effect of fasting on cholesterol biosynthesis in some biological systems has been established (26, 27).

There is a possibility that the results of the reported experiments might be influenced by the enzyme systems of the mollusc into which the radioactive cholesterol was injected. Mussels (*Modiolus demissus*) and clams (*Saxidomus giganteus*) have been injected with 4-C^{14}-cholesterol in salmon oil in the same manner as in these experiments and kept alive up to 28 days in an aquarium. On chromatography of the steryl azoylesters the portion of the chromatogram that corresponded to the middle zone of the starfish chromatogram contained less than 6% of the total activity in all zones. In one case this portion was acetylated and mixed with 17 parts of 7-cholestenyl acetate. The mixture lost

84.5% of its specific activity in only three crystallizations. It is therefore evident that by far the major part of the transformation was carried out by the starfish.

The radioactive 7-cholestenyl acetate from starfish was chromatographed on paper in a system that separates 7-cholestenyl acetate from 8(9)-cholestenyl acetate and 8(14)-cholestenyl acetate and also separates cholesteryl acetate from sitosteryl acetate. The 7-cholestenyl acetate retained its specific activity in this chromatogram. These results, together with the results from the crystallization of the 7-cholestenyl acetate, the 8(14)-cholestenyl acetate, prepared through acid hydrogenation, and the maleic anhydride treated product furnish proof for the identity of the radioactive material in the middle zone with 7-cholestenol.

Reports by several investigators have shown the presence in starfish of a number of sterols with differences in the sterol composition between species of the same class and sometimes within individual species (3, 4). It has been proved in the present investigation that cholesterol fed to a starfish will be efficiently converted to 7-cholestenol. If the assumption is made that the transformation is not limited to cholesterol but is carried out on Δ^5-sterols in general, then all the various Δ^5-sterols in the food will appear as corresponding Δ^7-sterols in the starfish. Thus the findings reported here offer an explanation for the diversity of marine sterols in regard to one group of marine invertebrates.

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ISOLATION AND IDENTIFICATION OF 11-KETOTESTOSTERONE IN SALMON PLASMA[1]

D. R. Idler, P. J. Schmidt, and A. P. Ronald

Abstract

A new blood steroid, 11-ketotestosterone, has been isolated from postspawned male sockeye salmon. The steroid was identified by chromatography, infrared spectra, chemical transformation, and synthesis. This steroid is present at a concentration of 12 μg/100 ml of plasma and probably was reported as corticosterone by other investigators. Evidence is presented in support of the occurrence of corticosterone at a very low concentration in salmon plasma. The concentrations of cortisol, cortisone, corticosterone, and total Porter–Silber positive steroids were determined in large plasma samples taken from postspawned fish of both sexes.

Introduction

A steroid with some of the properties of corticosterone is present (8 μg/100 ml) in the blood of the ray (1). Corticosterone was not detected in plasma of carp (2) or salmon from either sex (3) even though a substance with the properties of cortisol was readily detected. Plasma of both sexes of sockeye captured during spawning migration contained less than 1 μg/100 ml of corticosterone, and 6160 ml of plasma from prespawned females contained .05 μg/100 ml of a substance with the polarity of this steroid (4). However, others report that corticosterone is the principal steroid in the plasma of spawned male sockeye (5). The present study shows that 11-ketotestosterone is present in postspawned male sockeye plasma at a high concentration and would probably not be distinguished from corticosterone using the procedures employed by Phillips and co-workers (5).

Materials and Methods

Plasma of recently spawned male sockeye salmon, taken at Chilko Lake, British Columbia, in September, 1959, was processed to obtain the crude steroid fraction as previously described (6). Chromatography paper was prewashed (4) and steroid fractions and appropriate paper blanks were eluted with methanol by descending chromatography. The methylene chloride soluble steroid fraction, from each 800 ml of plasma, was streaked on 3.5 cm of paper and the chromatogram developed in hexane – 75% methanol to remove residual fat. Toluene – 70% methanol (TM-70) was then used to separate the steroids (7). The relative mobilities in toluene – 70% methanol (TM-70) of steroids found in salmon plasma and closely related steroids are listed in Table I. Other solvent systems used were heptane:benzene 1:1 – 70% methanol (HBM-70), toluene – 30% propylene glycol (TP-30), and heptane : benzene 1:1 – 30% propylene glycol (HBP-30).

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Contribution from the Fisheries Research Board of Canada, Chemistry Section of the Technological Station, Vancouver 8, B.C.

TABLE I

Relative chromatographic mobilities of steroids in toluene – 70% methanol

Steroid	Distance from origin (cm)*
Cortisol	5.3
Cortisone	9.5
11β-Hydroxytestosterone	12.5
Corticosterone	20.5
Reichstein's S†	20.5
11-Ketotestosterone	22.5
4-Pregnene-17α,20β-diol-3-one	25.4
4-Androstene-11β-ol-3,17-dione	27.0

*Distances were measured to the leading edges of the spots.
†11-Deoxy-17-hydroxycorticosterone.

Total plasma steroids with a dihydroxyacetone group were determined with a modified Silber–Porter method (8) and cortisone was measured by this method on the eluate from the TM-70 chromatogram. Cortisol was determined by acid fluorescence on the eluate from the TM-70 system, and corticosterone by the same method after rechromatography of the TM-70 eluate in HBM-70 (9, 10). 11-Ketotestosterone was quantitatively determined by ultraviolet absorption and alkali fluorescence (7) following chromatography in TM-70 and HBM-70. The acid-fluorescence curves for corticosterone were obtained on both TP-30 and HBM-70 eluates following rechromatography of the TM-70 eluate. All quantitative determinations were corrected according to the recovery of approximately equal amounts of pure compounds through the procedures. A total of 2400 ml of male and 1600 ml of female plasma was used in this study.

11-Ketotestosterone and the chromatographically identical plasma steroid (20 μg) were each treated for 40 minutes at 20° C with 15 μg of CrO_3 in 0.3 ml, ca. 98% acetic acid. The products were chromatographed in HBP-30 for 2.5 hours.

11-Ketotestosterone was prepared by $NaBH_4$ reduction of adrenosterone (11). Infrared spectra were recorded of micro KBr pellets using a Beckman IR-4 equipped with a beam condensing system.

Results

The unidentified steroid (11-ketotestosterone) from the plasma of males was only slightly less polar than corticosterone and "S" in TM-70 and would be nearly quantitatively included with these substances if the corticosterone area was cut out as recommended even when the solvent ran 38 cm (7). A complete resolution of 11-ketotestosterone from corticosterone and "S" was achieved in the HBM-70 system developed for 16 hours. A separation of corticosterone and "S" was best achieved in TP-30 run for 6 hours or longer.

The steroid from male plasma and authentic 11-ketotestosterone both had their absorption maxima at 238 mμ in methanol. Neither gave the typical blue tetrazolium reaction of equal amounts of α-ketols, thus eliminating such steroids as corticosterone. The unknown and 11-ketotestosterone could be

detected on paper chromatograms with a Zimmerman reagent and in solution they exhibited absorption maxima at 515 mμ. They had identical mobilities in the TM-70, HBM-70, and TP-30 solvent systems. Likewise, both substances gave identical sulphuric acid chromogens with maxima after 2 hours at 285 mμ, O.D. 0.435, and 355 mμ, O.D. 0.02, and minima at 230 and 335. These data agree with those of others (12, 13). Both substances were transformed by CrO_3 to a steroid which gave a positive Zimmerman reaction and had a chromatographic mobility identical with 4-androstene-3,11,17-trione which moved 20 cm in a solvent system which resolves it from closely related steroids. The infrared spectra leave no doubt as to the identity of the plasma steroid with 11-ketotestosterone (Fig. 1). There was 12 μg/100 ml of 11-ketotestosterone in male plasma.

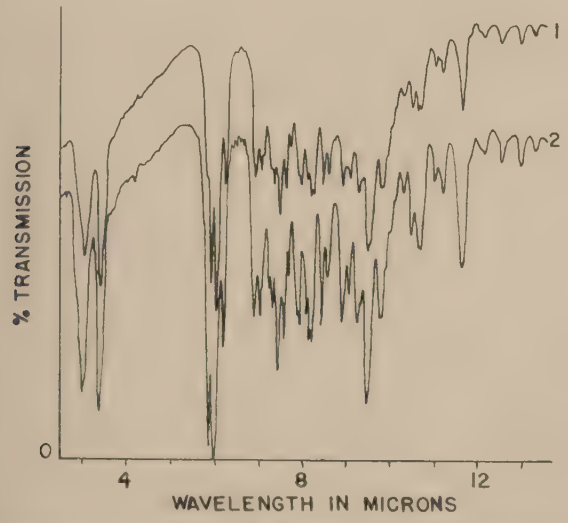

FIG. 1. Infrared spectra of (1) ca. 43 μg plasma steroid, (2) 80 μg 11-ketotestosterone.

An 870-ml sample of postspawned male plasma contained 32 μg/100 ml of steroid with the dihydroxyacetone group. Cortisol, cortisone, and corticosterone determinations on the same plasma gave 15.6, 13.6, and 0.27 μg/100 ml respectively. An 870-ml sample of postspawned female plasma contained 85.8, 36.3, 53.7, and .24 μg/100 ml respectively of each steroid.

The sulphuric acid–fluorescence curves of the corticosterone from plasma are compared with those obtained on the same quantity of corticosterone taken through the procedures (Fig. 2).

The "S" area of the chromatogram from 870 ml of male plasma showed a sharp maximum at 240 mμ equivalent to only 2.5 μg of steroid (ca. 50% recovery) or 0.6 μg/100 ml. This material will be pooled with other samples for further study.

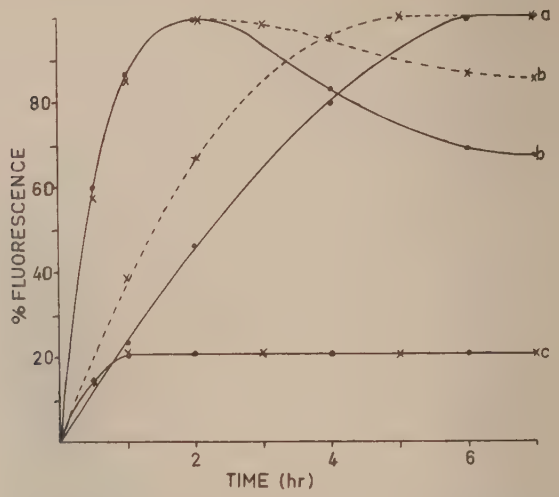

FIG. 2. Acid-fluorescence curves of corticosterone and plasma steroid eluted from paper chromatogram. (*a*) 6 H_2SO_4:4 ethanol. (*b*) 6.5 H_2SO_4:3.5 ethanol. (*c*) 9 H_2SO_4:1 ethanol. • Corticosterone. × Plasma eluate.

Discussion

Phillips *et al.* (4) report that the steroid with the mobility of corticosterone from 8868 ml of postspawned male sockeye salmon gave a positive triphenyltetrazolium reaction but the quantitative estimation was done by alkali fluorescence. 11-Ketotestosterone, on paper, does produce a chromogen of very low intensity with tetrazolium reagents. On the basis of the present report their sample could contain, depending on recovery, up to 24 μg of corticosterone and 40 μg of "S", which should be adequate to explain the positive TPZ reaction. Towards the completion of this study a report appeared describing corticosteroid production by interrenal tissue of teleost fishes (14). These authors mentioned a substance which was detected with TPZ and was slightly less polar than corticosterone, but no other properties were reported.

Acid fluorescence is quite selective and no other steroid has been found that fluoresces and gives the time curves of corticosterone (10). Considering the small amount of plasma steroid available we believe that the agreement with the curves of corticosterone, as well as the mobility in three solvent systems, strongly suggests the identity of the two. 11-Ketotestosterone does not acid-fluoresce under the conditions for the determination of corticosterone and "S" has only 0.8% of the fluorescence of corticosterone. Both would interfere with the determination of corticosterone by alkali fluorescence.

It is of interest that there was no detectable 11β-hydroxytestosterone in the male plasma since this steroid is produced from testosterone by both adrenal (12) and testicular tumor tissue (15). The origin and significance of 11-ketotestosterone is being investigated. To our knowledge this is the first reported isolation of this steroid from blood or other natural product.

Acknowledgment

The staff of the International Pacific Fisheries Commission kindly assisted with the sampling.

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THE METABOLISM OF INOSITOL IN SALMON

I. THE DISTRIBUTION OF RADIOACTIVITY IN ACID-SOLUBLE EXTRACTS OF COHO SALMON FOLLOWING INTRAPERITONEAL AND INTRA-ARTERIAL INJECTIONS OF 2-C^{14}-MYOINOSITOL[1]

H. Tsuyuki and D. R. Idler

Abstract

Male coho salmon held in captivity for only a few days were injected intraperitoneally and intra-arterially with 2-C^{14}-myoinositol. Several hours later the cohoes were anesthetized and frozen in dry ice. After overnight storage, perchloric extracts were made of the major organs and tissues and the relative C^{14} content of the extracts was determined. Most of the radioactivity of the extracts was not adsorbed by charcoal. Only the liver and to a lesser extent the flesh and kidney contained radioactivity in the nucleotide fraction. Of the material not adsorbed to charcoal, most of the radioactivity of the kidney, milt, and gut extracts were not held by Dowex-1-formate while that of the head and liver extracts was about equally divided between the water and formic acid eluants of the column. The two methods of injection resulted in no major differences in the relative amounts of C^{14} labelling in the various tissue components.

Introduction

An extensive study of the distribution of total acid-soluble inositol in the various tissues of the sockeye salmon (*Oncorhynchus nerka*) has shown a low of about 150 μg in the head, skin, and bones to a high of about 600 μg per g of liver tissue (1). In order to study the pathway of inositol metabolism in the salmon and to select the best tissue for this study, the distribution of radioactivity in the various tissues of the coho (*Oncorhynchus kisutch*) following introduction of 2-C^{14}-myoinositol was investigated.

Methods

*Intraperitoneal Injection of 2-C^{14}-Myoinositol**

A 2340-g male coho (C^{14}-coho 1) held in fresh-water captivity was anesthetized in 30 liters of fresh water containing 30 ml of quinaldine (2) (100 mg per ml of acetone). After 30 minutes, 3.98 mg (22 μc total activity) of 2-C^{14}-myoinositol in 2.0 ml of physiological saline was injected intraperitoneally. Seven hours later the tail was removed and the fish bled. The liver was excised and the remainder of the fish frozen immediately in dry ice together with the liver and the blood. After overnight thawing in the refrigerator, the carcass was separated into kidney, milt, guts, heart, spleen, flesh, head, skin, and bone and tail (Table I).

An untreated 1860-g male coho was also prepared in the same manner. Due to the small amount of the sample, the liver, kidney, guts, and heart tissues from the two fishes were combined (Table I).

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*2-C^{14}-myoinositol was kindly supplied by Prof. Laurens Anderson of the University of Wisconsin.

TABLE I

Weights of the tissues and distribution of radioactivity

Tissue	Weight (g)		Total c.p.m.†	
	C^{14}-coho I	C^{14}-coho II	C^{14}-coho I	C^{14}-coho II
Head	370	—	5,875	—
Heart*	14.3	9.4	375	2,772
Blood	39.3	65	1,535	25,320
Skin–bone–tail	405	—	28,020	—
Flesh	1,314	—	5,250	—
Liver*	41.4	48	9,000	20,450
Kidney*	43.0	46.5	106,000	58,340
Milt	119	197	75,900	52,200
Gut*	51.5	75.5	262,000	28,200
Spleen	3.0	9.0	—	2,420

*Represents the combined weights from C^{14}-coho I and untreated coho. The following amounts of tissue from untreated coho were added: heart, 8.8 g; liver, 17.9 g; kidney, 17.6 g; gut, 18.0 g.
†A Nuclear-Chicago instrument was used to determine radioactivity.

Intra-arterial Injection of 2-C^{14}-Myoinositol

2-C^{14}-Myoinositol (3.65 mg (20.3 μc total activity)) was injected into the dorsal aorta in the roof of the mouth between the third and fourth gill arches (3) of a 4125-g male coho (C^{14}-coho II) in three 1.0-ml doses at intervals of $2\frac{1}{2}$ hours. Each dose was followed immediately by an intraperitoneal injection of 5.0 ml of sterile 50% aqueous glucose solution. Anesthesia was administered by holding the fish head first for a few minutes into a large cylinder containing 300 mg of MS-222 (tricaine methanesulphonate) in 3 liters of water. Four and one-half hours after the final set of injections, the fish was killed and the various tissues separated and stored as previously (Table I).

Acid-soluble Extracts

Perchloric acid soluble extracts of the various tissues were prepared by the method of Hurlbert *et al.* (4) modified slightly (5).

Results

Distribution of Radioactivity in the Tissues

Suitable aliquots of the perchloric acid extracts were counted and calculated on the basis of total counts per minute (c.p.m.), above an average background of 13.5 c.p.m., in the respective tissues of the salmon (Table I). As would be expected the intra-arterially injected C^{14}-coho II shows relatively much higher distribution of activity in the blood and the tissues normally concerned primarily with the vascular system of the fish. The higher total activity found in the liver of C^{14}-coho II compared to that of the combined livers of C^{14}-coho I and the untreated coho is readily explained by the fact that the former liver is more than twice the weight of the undiluted latter liver. The high activity of the kidney and guts of C^{14}-coho I may well be due to unchanged 2-C^{14}-myoinositol which would be present in this region of the fish where the intraperitoneal injection was carried out. Perchloric extracts of head, skin, and bone and tail contained a high solid residue and the counts were only approximate.

Charcoal Separation of Perchloric Extracts

Two-milliliter aliquots of acid-soluble extracts of C^{14}-coho I were put on a

previously washed 1-g column of Norit* A, N.F., IX:celite (1:1) and eluted with 100 ml of water followed by 100 ml of ethanol:NH_3:water (40:1:59). The charcoal column technique has been proved satisfactory for the separation of nucleotides from non-nucleotide phosphate compounds of acid extracts of rat skeletal muscle by other workers (6). Inositol monophosphate from rat and guinea pig liver extracts is not adsorbed to charcoal (7).

It was found that previous washing of the 1-g charcoal column with 20 ml of water was sufficient to remove all the residual ultraviolet-absorbing material. Forty milliliters of water eluted virtually all the inorganic and non-nucleotide phosphates and 40 ml of ethanol:NH_3:water eluted almost all the material absorbing at 260 mμ. The ethanol:NH_3 and the water fractions were concentrated by means of a dry ice – acetone trapped rotary evaporator at 40° C in a 50-ml round-bottom flask and transferred to 5.0-ml volumetric flasks. The activity was counted on an aliquot and calculated on the basis of total activity in the tissue (Table II). Of the tissue extracts subjected to this treatment,

TABLE II

Separation of C^{14} activity of coho I perchloric acid extracts by charcoal chromatography

Tissue	Charcoal column eluate	
	Water wash (c.p.m.)	Alcohol:NH_3:water* (c.p.m.)
Head	7,860	—
Skin–bone–tail	35,600	—
Flesh	1,950	240
Kidney	77,500	700
Liver	6,325	2,880
Milt	57,500	—
Gut	191,000	—

*An insignificant amount of C^{14}-activity was found in the charcoal alcohol:NH_3 eluates of head, skin–bone–tail, milt, and gut.

only the liver, and to a much lesser degree, flesh and kidney, showed sufficient activity in the nucleotide fraction to warrant further investigation. Most of the activity in the other tissues was found in the non-nucleotide fraction. In the kidney, milt, and gut tissues about 25% of the original activity could not be recovered from the charcoal columns under the conditions employed. No attempt was made to ascertain the nature of compounds responsible for the activity retained by the charcoal. The perchloric acid extracts of heart and blood from C^{14}-coho I showed so little activity that they were not subjected to the charcoal treatment.

Treatment of C^{14}-coho I Charcoal Water Wash on Dowex-1-Formate

The water washes from charcoal:celite columns were separated into broad groups of compounds by passing through a 1 × 20 cm column of Dowex-1-formate and eluting with 500 ml of water followed successively with 150 ml of 0.4 *N* and 350 ml of 4.0 *N* formic acid solutions (Table III). In other experiments, inositol and free sugars were eluted with water immediately after the

*Obtained from Pfanstiehl Laboratories, Inc.

TABLE III

Separation of C^{14} radioactivity from coho I charcoal water washes by Dowex-1-formate

Tissue	Water wash (c.p.m.)	Dowex column eluates: Formic acid (c.p.m.) 0.4 *N*	4.0 *N*
Head	1,920	860	—
Liver	2,340	3,610	—
Kidney	78,800	1,500	1,100
Milt	56,800	—	747
Gut	208,000	575	—

resin-bed volume of water had come through. Uronic acids were not eluted until after 50–60 ml of 0.4 *N* formic acid was passed through. Inositol monophosphate has been shown to elute by the gradient elution system of Hurlbert *et al.* (4) when the formic acid reached a calculated concentration of 1.38 *N* in the peak tube, and the hexose phosphates at a concentration of 1.69 *N*. However, in this initial experiment only the kidney and milt appear to show any activity in the inositol phosphate and sugar phosphate fractions. Liver tissue does not appear to have any activity in these phosphates (Table III). However, the more heavily labelled C^{14}-coho II liver tissue should be investigated in this respect since both these phosphates were found in the livers of female coho salmon captured at sea.

Discussion

The two methods of introducing 2-C^{14}-myoinositol into the coho do not show a great deal of difference with respect to the concentration of C^{14} in the various tissues other than the blood and the alimentary tract, both of which were sites of injections. The intraperitoneal introduction of glucose following intra-arterial injection of 2-C^{14}-myoinositol did not strikingly increase the over-all labelling in the liver as has been shown for liver glycogen in the rat (8).

Of all the salmon tissues, the liver showed the highest proportion of activity in the nucleotide fraction and since extensive studies of the nucleotides of this tissue have already been carried out (5), part II of this series will describe the results on liver nucleotides.

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THE METABOLISM OF INOSITOL IN SALMON

II. THE ROLE OF NUCLEOTIDES IN RELATION TO THE METABOLISM OF 2-C^{14}-MYOINOSITOL IN COHO LIVER[1]

H. Tsuyuki and D. R. Idler

Abstract

Though radioactivity is distributed throughout the entire spectrum of coho liver nucleotide fractions, the UDP-uronic acid fraction was found to be one of the more active. Most of the radioactivity was present in the glucuronic acid and glucuronolactone formed when UDP-uronic acid was hydrolyzed with acid. UDP-glucose, UDP-galactose, and UDP-N-acetylhexosamine did not contain any measurable activity. In C^{14}-coho II another major concentration of activity was found in fraction A_8, which has not yet been investigated. These results are discussed in relation to the known biochemical reactions of inositol and sugar nucleotide interconversions in plants.

Introduction

The conversion of deuterium-labelled myoinositol to urinary glucose in the phlorizinized rat was first demonstrated by Stetten and Stetten (1). Subsequently, Posternak (2) showed that 2-deuterated myoinositol was converted almost exclusively to 6-deuterated urinary glucose. Direct ring opening of the inositol to form glucose was not deemed possible since the deuterium would then be found in the 5 position of glucose. Anderson and Coots (3) found over 80% of the label from 2-C^{14}-myoinositol in positions 1 and 6 of glucose obtained from the hydrolysis of rat liver glycogen. The opposite experiment, the conversion of C^{14}-labelled glucose to C^{14}-myoinositol, has been demonstrated by Halliday and Anderson (4) and by Daughaday *et al.* (5).

The distribution of the C^{14} label and the average specific activity of the urinary glucuronic acid carbon, which was sevenfold greater than that of glycogen, indicated that glucuronic acid could not have arisen entirely from the oxidation of glucose but was derived in part more directly from the labelled inositol (3). Partially purified rat kidney enzyme preparations converted myoinositol to DL-glucuronic acid (6). Degradation of the glucuronate formed by incubation of 2-C^{14}-myoinositol with this enzyme system revealed that essentially all of the label appeared in the 5 position of glucuronate, thus showing cleavage between the 1 and 6 carbons of inositol (7).

The pathways concerned in myoinositol metabolism have been schematized by several workers on the basis of data obtained by tracer experiments (8, 9, 10). It is generally agreed that D-glucuronic acid is among the first metabolites of inositol.

In the present paper evidence is presented showing that, of the nucleotides from salmon liver acid extracts, uridinediphosphoglucuronic acid (UDP-glucuronic acid) is heavily labelled in the glucuronic acid moiety following intraperitoneal and intra-arterial injections of 2-C^{14}-myoinositol.

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Methods

Preparation and Column Chromatography of Samples

The perchloric extracts of livers (11) were separated at 0° C into various nucleotide fractions on columns of Dowex-1-formate following a gradient elution method described previously (12). A 1.4×20 cm column was used to separate the liver extract of C^{14}-coho I. The column was washed first with 300 ml water and then developed successively with 1000 ml of 4 *N* formic acid, 1000 ml of 4 *N* formic acid and 0.2 *M* ammonium formate, 1000 ml of 4 *N* formic acid and 0.4 *M* ammonium formate, and finally with 4 *N* formic acid and 2.0 *M* ammonium formate till no further ultraviolet-absorbing material was eluted. Ten-milliliter fractions were collected and a 1000-ml mixing volume was used. Fraction I was rechromatographed on a 1×20 cm Dowex-1-formate column. A 500-ml mixing volume was used and the column developed successively with 500 ml of 1 *M* ammonium formate, 500 ml of 2 *M* ammonium formate, and a mixture of 2 *M* ammonium formate and 0.75 formic acid until there was no further elution of ultraviolet-absorbing material. Five-milliliter fractions were collected. Liver extract of C^{14}-coho II was fractionated by a longer, 1.4×40 cm, column to obtain better separation of the weakly bound components. The column was washed thoroughly with 1000 ml of water and developed exactly as described above.

Where ammonium formate was present in the eluates the solvent was removed as far as possible by means of a rotary evaporator trapped with dry ice – acetone mixture and evacuated by an oil pump. It was possible to remove water at a very rapid rate by this method while holding the temperature of the sample near freezing. After most of the water and formic acid were removed in this manner the residue was redissolved in 200–300 ml of water and then ion-exchanged with Amberlite IR-120 at 2° C. The eluates were then concentrated to dryness, redissolved in about 25 ml of water, and again concentrated to remove traces of formic acid. The residue was taken up in 10 ml of water and stored at −30° C.

Paper Chromatography

Chromatographic grade of Whatman No. 1 sheets were used. The chromatograms were developed at 20° C in a descending fashion. The solvent systems employed were as follows: solvent 1, isobutyric acid:ammonium hydroxide: water, 66:1:33; solvent 2, 0.1 *M* phosphate (pH 6.8):ammonium sulphate (600 g dissolved in 1 liter of 0.1 *M*, pH 6.8, sodium phosphate buffer): *n*-propanol, 100:60:2; solvent 3, *n*-butanol:pyridine:water, 6:4:1; solvent 4, *n*-butanol:acetic acid:water, 4:1:5.

Paper Electrophoresis

Electrophoresis was carried out on oxalic-acid-washed Whatman No. 1 paper in 0.1 *M* ammonium formate, pH 3.6, at 900 volts for 2 hours and 45 minutes (13).

Location of Sample on Chromatograms

Ultraviolet-absorbing samples were located with a mineralight lamp (Ultra-Violet Products Inc.), the uronic acids and hexoses by spraying with either

aniline phthalate (14) or ammoniacal silver nitrate, which was also used for glucuronolactone and L-gulonolactone. Acetylhexosamine was located by the modified Elson-Morgan reagent (15).

Analytical Methods

Hexoses were determined by the Folin and Malmros (16) method, the uronic acids by a modification of the method of Meijbaum (6), and total phosphorus by the method of Bartlett (17). The concentration of UDP-uronic acid and UDP-N-acetylhexosamine were determined by absorption at 261 mμ, using $E = 9.7 \times 10^3$ for uridylic acid at pH 1.0. The absorption was read against an appropriate paper blank eluted with same strength acid. Inositol was determined by a slight modification (18) of the yeast assay method using *Saccharomyces carlsbergensis* A.T.C.C. 4228 (19).

Radioactivity Determinations

Radioactivity was determined on a Nuclear-Chicago counter equipped with either a Geiger–Mueller tube or flow-gas attachment. Activity bands on paper strips were located with a Nuclear-Chicago actigraph II, Model C-100 A.

Results

Distribution of Radioactivity in Dowex Column Eluates

One-tenth-milliliter aliquots of the perchloric extracts were plated and counted. The results were expressed as total counts per minute (c.p.m.) in the entire fractions (Table I). Liver extract of C^{14}-coho II shows two major

TABLE I

Distribution of radioactivity in the nucleotide fraction of coho liver

Fraction	Total c.p.m.	
	C^{14}-coho I*	C^{14}-coho II†
Water wash (1)‡	1,640‖	9,270
Water wash (2)§		15
A_1		150
A_2		1,120
A_3	1,070¶	3,380
A_4		446
A_5		55,700
B	320	434
C	—	319
D	—	256
E	—	1,280
F	—	1,450
G	—	599
H_1	—	4,330
H_2	—	2,000
I	330	64,800
J and K	90	702
Beyond K	—	563

*Separated by a 1.4×20 cm column of Dowex-1-formate.
†Separated by a 1.4×40 cm column of Dowex-1-formate. All counts for samples from C^{14}-coho II were carried out on a Nuclear-Chicago counter equipped with flow gas and the average c.p.m. is 5.2 times higher than all previous counts.
‡First 400 ml water wash.
§Remaining 600 ml water wash.
‖Activity found in the total water wash (300 ml for this liver sample).
¶Total activity found in the A fractions, A_1 to A_5, inclusive.

concentrations of activity, fractions 1 and A_5, which coincided with ultraviolet absorption. The activity in the water washes was shown chromatographically in solvent system 4 to be chiefly unchanged 2-C^{14}-myoinositol in C^{14}-coho I. By a microbiological method free inositol was also found repeatedly in the water washes of Dowex-1-formate columns. Some activity was found in glucose and very little in the glycogen.

Fraction I from C^{14}-coho II

When an aliquot of fraction I, representing 5550 c.p.m., was chromatographed in solvent 1 for 18 hours, one of the ultraviolet-absorbing spots moved like UDP-glucuronic acid reference standard as found previously. The UDP-uronic acid spot corresponded exactly with the radioactivity band when the strip of paper was passed through an actigraph. When the remainder of the chromatogram was cut into sections, eluted, and counted, a little over 1% of the total initial activity was found, indicating that virtually all the activity was present in the UDP-uronic acid component. Long-period paper chromatography in solvent 1 as well as paper electrophoresis failed to separate this radioactive nucleotide from UDP-glucuronic acid reference standard.

The distribution of radioactivity in the UDP-uronic acid and its hydrolysis products are shown in Table II. The nucleotide band was eluted, hydrolyzed in 1.0 *N* HCl for 30 minutes in a boiling water bath, and the hydrolyzate

TABLE II

Chromatographic separation of activity in fraction I

Compound	Fraction I C^{14}-coho II*		
	Expt. 1 (c.p.m.)	Expt. 2 (c.p.m.)	Expt. 3 (c.p.m.)
(*a*) UDP-uronics†	4285	4122	—
(*b*) Uronic acid	2333	1459	2290
(*c*) Glucuronolactone	1098	2505	3058
(*d*) % recovery of original activity in (*b*) + (*c*)	62	71.3	96.5

*A total of 5500 c.p.m. was separated in each experiment.
†In another experiment 95% of the original activity was recovered in UDP-uronic acid after it had been separated in solvent 1 for 116 hours. In experiment 3 the original sample was hydrolyzed directly.

rechromatographed in solvent 1 to determine the activity in the uronic acids and the glucuronolactone. Under the conditions of hydrolysis, glucuronic acid is largely lactonized (about two-thirds) to glucuronolactone, though an equilibrium is reached. Approximately 75 to 95% of the original activity was recovered in the UDP-uronic acid band. In the experiments with low recovery some of the counts must have been lost during the numerous elutions and transfers required to prepare the samples for counting, for in another experiment 97% of the initial activity was recovered in the uronic acid and lactone bands when the sample was hydrolyzed prior to chromatography.

When the uronic acid band obtained by hydrolysis was passed through Amberlite IR-120 at room temperature to remove ammonium ions and chromatographed in solvent 1 for 6 days, about three times more activity ran off the

paper than was recovered in the glucuronic acid region. The region of the chromatogram corresponding to galacturonic acid reference standard had little or no activity and showed a negative silver nitrate spot test. The active material which ran off the paper proved to be glucuronolactone when chromatographed using solvent 1 for 3 days. This indicated that most of the activity found in the uronic acid following acid hydrolysis of UDP-uronic acid was due to glucuronic acid which subsequently lactonized upon treatment with Amberlite IR-120 at room temperature. Galacturonic acid does not lactonize in acid. Furthermore, the activity of glucuronolactone separated into glucuronic acid and its lactone in a ratio of about 1 to 3 when passed through a sulphonic resin column. The radioactive uronic acid moiety of the UDP-uronic acid in fraction I is therefore chiefly glucuronic acid.

L-Gulonic acid has been found by other workers to be an intermediate in inositol metabolism (10). This acid lactonizes readily (20) and its lactone moves a little slower than glucuronolactone in solvent 1 when run for 40 hours. Little or no activity was found in the L-gulonolactone band.

Specific Activities

After acid hydrolysis of the UDP-uronic acid, the unlactonized glucuronic acid band was eluted from the chromatogram and estimated quantitatively along with a paper blank of identical size from the adjacent region of the paper. The radioactivity was determined on an aliquot of the same sample. The specific activity of glucuronic acid from C^{14}-coho II is slightly higher than that of UDP-uronic acid (Table III) indicating that small amounts of other acids, of

TABLE III

Specific activities of UDP-glucuronic acid and glucuronic acid released by acid hydrolysis

Compound	Activity per μmole	
	C^{14}-coho I (c.p.m.)	C^{14}-coho II (c.p.m.)
Glucuronic	675	2,370
UDP-uronic acid*	—	1,983

*The specific activity of UDP-uronic acid of C^{14}-coho I was not determined.

little or no activity, may be present in the UDP-uronic acid mixture. The lower specific activity of glucuronic acid from C^{14}-coho I is due partly to the fact that this liver tissue is a composite of 23.5 g of radioactive liver and 17.9 g of non-radioactive liver (11).

Fraction G of C^{14}-coho II

This fraction, which moved like UDP-hexose in solvent 2, was hydrolyzed in 0.1 *N* HCl at 100° C for 20 minutes, ion-exchanged through a mixed bed column of Amberlite IR-120 and IR-45, and separated in solvent 3 for 40 hours. With an ammoniacal silver nitrate spray the galactose band was obtained as previously shown (12) and in addition a glucose band was also found, confirming the observations of Forrest and Hansen (21). The sugars eluted from a 40-hour chromatogram in solvent 3, and analyzed along with appropriate blanks,

showed values of 0.33 μmole of galactose and 0.533 μmole of glucose in fraction G, indicating that 38% of the UDP-hexose was UDP-galactose and 62% UDP-glucose. A higher glucose content was expected since the fish had been injected with large doses of glucose (11). In other UDP-hexose fractions of female cohoes captured at sea, galactose contents of 19 and 40% were found. Glucose was also found in fraction G of spring salmon liver investigated earlier (12).

Of the 600 c.p.m. present in fraction G (Table I), 71.5% of the activity has been recovered from the various regions of the sugar chromatogram. However, very little, if any, radioactivity was found in the glucose and galactose region of the paper, indicating that UDP-glucose and UDP-galactose are not early transformation products of inositol.

Fraction F of C^{14}-coho II

Fraction F, which moves like UDP-N-acetylhexosamine in solvent 2, and which precedes UDP-hexoses during column chromatography (12), was hydrolyzed in 0.1 *N* HCl for 30 minutes at 100° C. When the hydrolyzate, representing 5.6% of the entire fraction F, and containing 0.215 μmole, was separated in solvent 3 for 40 hours, the band corresponding to N-acetyl-glucosamine reference standard showed no activity.

Discussion

The radioactivity of acid-soluble phosphorus compounds of coho liver is found mostly in fraction A_5 and in the glucuronic acid moiety of UDP-glucuronic acid following introduction of 2-C^{14}-myoinositol. On the basis of known interconversions of sugar nucleotides in plants, summarized by Hassid *et al.* (22), one of the biosynthetic sequences by which UDP-glucuronic acid is formed is as follows:

$$\text{D-glucuronic acid} \xrightarrow{\text{ATP}} \alpha\text{-D-glucuronic acid-1-phosphate} \underset{\text{PP}}{\overset{\text{UTP}}{\rightleftharpoons}} \text{UDP-D-glucuronic acid}$$

This series of reactions is probably also the route by which inositol is converted to UDP-glucuronic acid, since D-glucuronic acid is generally considered to be one of the first products formed after the ring opening of inositol between the 1 and 6 carbon atoms (3, 6, 8, 9). The absence of measurable activity in UDP-glucose might be explained by the apparent irreversible nature of the conversion of UDP-glucose to UDP-glucuronic acid (23, 24) and by the fact that inositol must go through the pentose cycle (10) to form glucose, which must then be phosphorylated and finally converted to UDP-glucose. The many dilutions involved in such a reaction series could conceivably dilute the C^{14} to a point where it becomes difficult to measure.

Further work is in progress to identify other active fractions, particularly A_5, in the acid extracts of salmon liver.

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ARCTIC INSTITUTE OF NORTH AMERICA

TECHNICAL PAPER NO. 5

ARINE INFAUNAL BENTHOS IN ARCTIC NORTH AMERICA

By

DEREK V. ELLIS

PUBLISHED SEPTEMBER 1960 FRB No. 623

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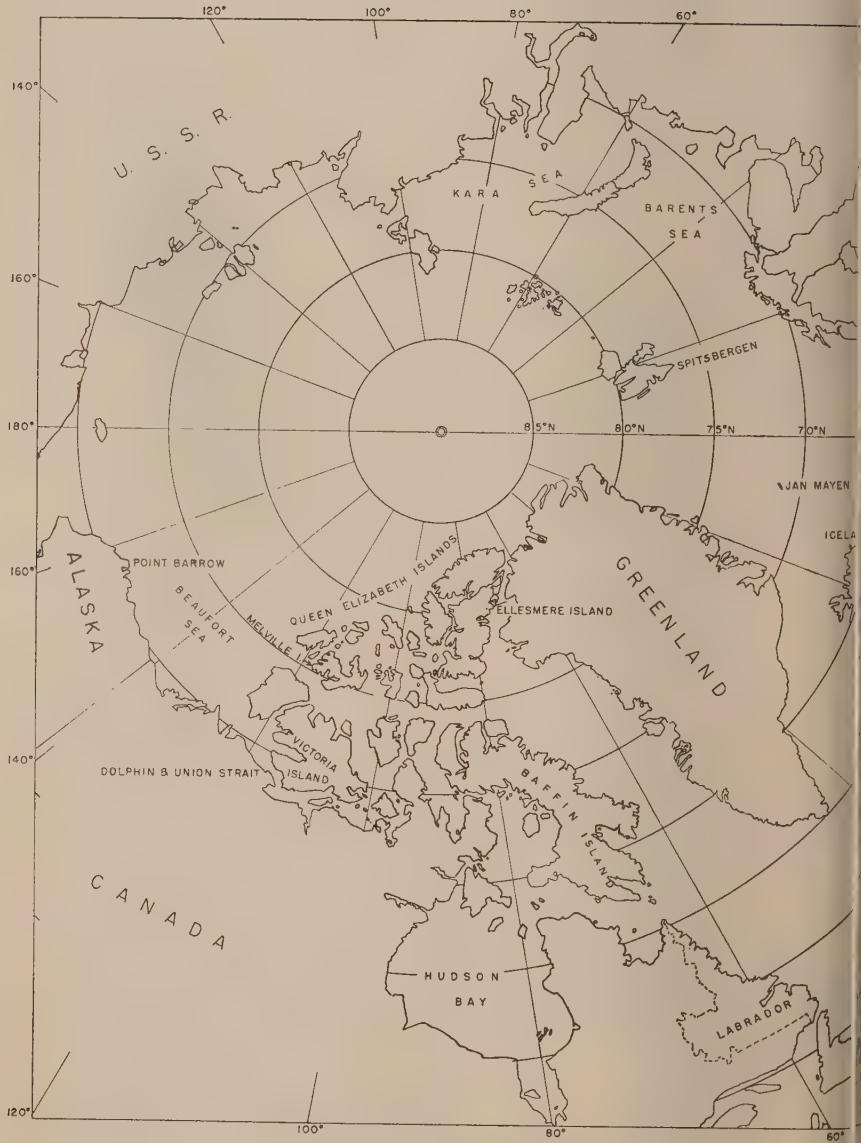


Fig. 1. Arctic North America.

I E INFAUNAL BENTHOS IN ARCTIC NORTH AMERICA

Derek V. Ellis

I. Introduction

E have been many attempts to measure the crop of animals on the sea tom in north European waters, where much of the benthos is important of food for commercial fish. Although benthic crops have also been d in a few arctic localities, notably the Barents Sea and Greenland, no e previously been available from arctic Canada. In this region the enthos is eaten by several species of mammals, sea birds, and fish, for walrus, bearded seals, eider ducks, flounders, and rock cod, which are potential commercial value or of actual economic importance to the skimo. A knowledge of the food supply available to these benthic s is part of the information required for their efficient management. order to estimate standing crops of benthos in arctic Canada, and to rate general features of benthic distribution and abundance in the American Arctic, quantitative surveys were made over a period of four 954–7, in northern Baffin Island, west Greenland, and northwest Foxe igs. 1 and 2). The general term "arctic North America" when used aper refers to east and west Greenland, arctic Canada, and also arctic

e marine benthos can be arbitrarily separated into infauna, animals living level (soft) sea bottom, and epifauna, animals living on or attached surfaces. The distribution and standing crops of infaunal species are d here, using the concept of marine level-bottom communities (Thor-7), which is a particularly useful way of summarizing the infauna of egions. The distribution of epifaunal species collected is shown in the x.

II. Methods and Materials

e surveys were started near Arctic Bay and Pond Inlet settlements, north sland, during 1954 and 1955 (Fig. 2). They were continued during two laboratories in Greenland to obtain increased accuracy by working cilities adequate for examination of fresh specimens. These were the Biology Laboratory, Godthaab, and the Arctic Station of the University enhagen, Godhavn (Fig. 2). During 1956 Greenland's Fisheries Investi- permitted me to examine some of their benthos collections from the grounds in Disko Bugt.

e Arctic Unit of the Fisheries Research Board of Canada turned over for examination benthos collected by dredges near Igloolik, Foxe Basin, and 1956, and in 1957 the same unit provided me with the opportunity ct more benthos in Frustration Bay, Rowley Island (Fig. 2), while I was

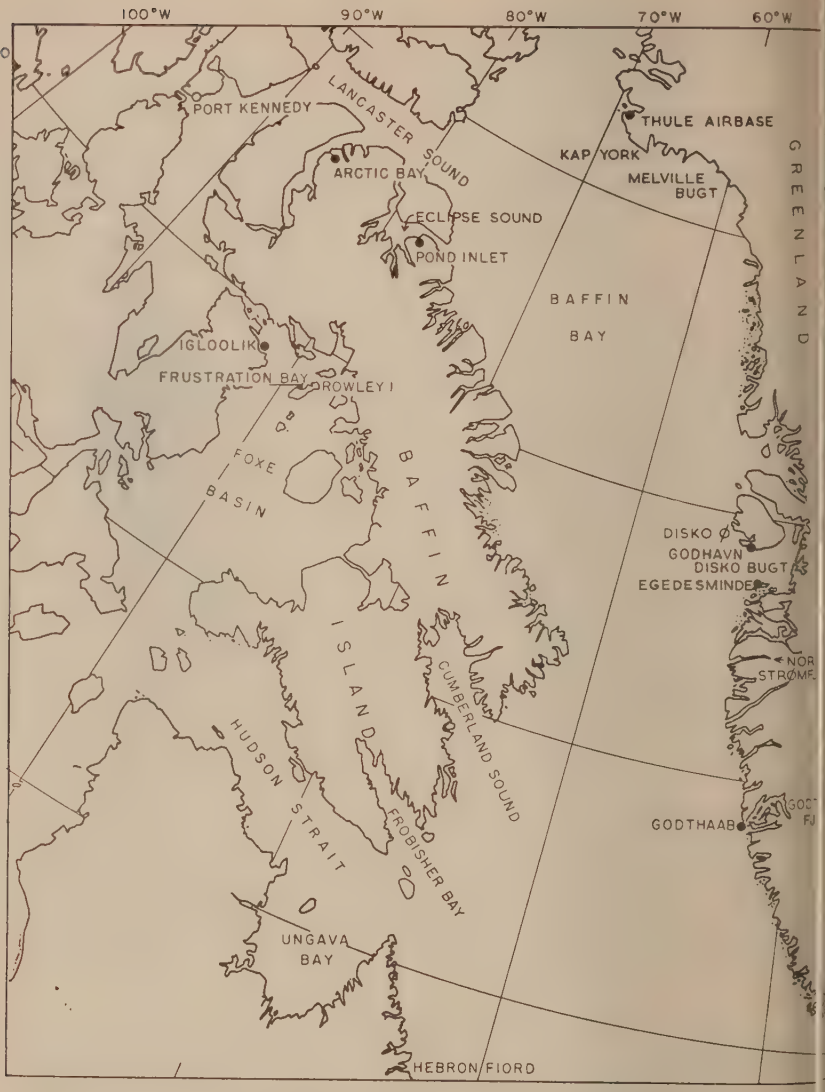


Fig. 2. Eastern arctic North America.

working on board C.G.M.V. *Calanus* from May 19 to August 30. The pu
and programs of the *Calanus* expeditions are described by Dunbar (195
in the *Annual Reports* of the Fisheries Research Board of Canada.

1. Gear and collecting techniques

Two types of grab were used in the surveys. The first, made by M
Foerst, Chicago, sampled a surface area of 0.07 sq. m. and a maximum v
of 5 litres (Pl. 1). It was used in Baffin Island. This grab was similar

Pl. 1. Foerst grab with a haul of benthos being pulled on to sea ice in Eclipse Sou

Pl. 2. Van Veen grab, in open position, on the *Adolf Jensen* in Godthaab Fjord.

n dredge" figured in Welch (1948), but differed greatly from the Petersen grab (Ursin, 1954). The second, a Van Veen grab, sampled . of bottom and a maximum volume of 15 litres (Pl. 2). It was used nland and in Foxe Basin. The Van Veen grab was described by up (1938) but the models used in these investigations were slightly (Ursin, 1954 and 1956).

Baffin Island only hauls more than half-full (2.5 litres) were used for ive records. In Greenland the volume of all hauls was measured. At ab, where many hauls were made, only the largest, 4–6 litres, have been standing crop estimates. In Disko Bugt and also in Foxe Basin hauls nerally between 4 and 6 litres and smaller hauls were rejected for ing the quantitative data. In Egedesminde harbour soft mud bottom d taking hauls of maximum volume (15 litres).

Baffin Island a sieve with a 1.5-mm. mesh was used to separate animals diment; at Godthaab a 2-mm. mesh was used, at Godhavn a 1-mm. nd in Foxe Basin a 2-mm. mesh. These mesh sizes correspond to those similar surveys elsewhere, and the use of different-sized meshes does parently affect the results described here. The animals retained by the re equivalent to the "macrobenthos" and larger "meiobenthos" of Mare but not to her smaller "meiobenthos" and "microbenthos" nor to the fauna of the bottom" of Smidt (1951).

Baffin Island the animals in each haul were preserved in 4 per cent zed formalin and were later counted and weighed. Specimens in 32 ere also weighed before preserving. The preserved weights from these ave been divided by the equivalent fresh weights to give a coefficient ert preserved weights to fresh weights. There was on the average a cent loss in weight through preservation and the conversion coefficient In Greenland and Foxe Basin specimens were counted and weighed preservation, except for 5 hauls from the prawn grounds in Disko Bugt. hauls were made in 1953 by Greenland's Fisheries Investigations and nained in preservation for three years before I examined them. Preserved s of these specimens have also been multiplied by 1.2 to give fresh s, using the same conversion coefficient as for the Baffin material.

he convention of converting numbers and weights of animals per haul lues per sq. m. has been followed here to facilitate comparison with ative surveys from other areas (Thorson, 1957).

2. Areas surveyed

Baffin Island

The survey was made in two parts: in Arctic Bay (Fig. 3) between September and 1 December 1954, and in Eclipse Sound (Fig. 4) near Pond Settlement between 16 June and 28 July 1955. When the sea was free the grab was operated from a small dinghy but otherwise the grab was ed through cracks or holes in the sea ice.

The collections were arranged in a series of 6 sections (plus a few extra extending out from shore approximately perpendicular to the coast. n 1 in Arctic Bay revealed mud bottom at 7 metres with large numbers es, which gradually became less numerous with increasing depth (Fig. 3). aximum depth sampled was 48 metres. There were several small streams

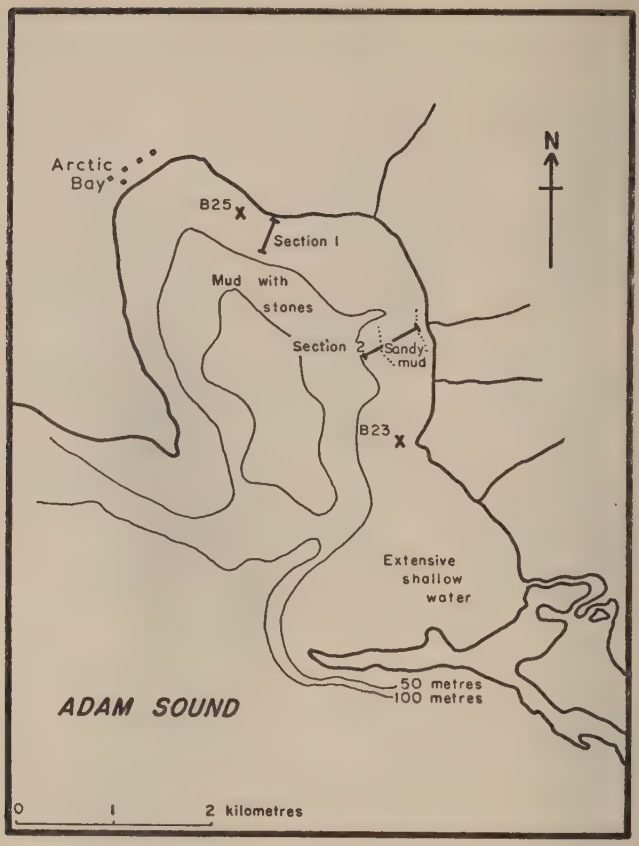


Fig. 3. Collectin grounds in Arctic Bay, Adam Sound

draining into the bay along its eastern shore and section 2 showed that t bottom near the mouths of at least one of these small streams consisted of sa in very shallow water (about 2 metres depth), changing gradually with incre ing depth to sandy-mud (about 7 metres depth), and then to mud (at abo 30 metres depth).

A narrow zone of sand stretched along the shore near Pond Inlet (Fig. Section 3 at a small point east of the settlement showed that the sand grad into sandy-mud below 14 metres depth; hauls were taken to a maximum dep of 47 metres. There was a sandy submerged delta at the mouth of the Salm River west of Pond Inlet settlement and sections 4, 5, and 6 crossed this de to 43, 55, and 11 metres respectively. The sand was replaced by sandy-m or mud between 5 and 10 metres depth in the three sections. In many of t hauls at the mouth of the river there were small pieces of coal which had be washed down by the Salmon River from deposits upstream.

b. Godthaab Fjord

In the Godthaab survey from 9 May to 6 June 1956, the usual method making grab collections from a ship was modified so that the animals in hauls could be examined alive. The Van Veen grab was operated from t

4. Collecting
ds in Eclipse
d near Pond
settlement.

enland's Fisheries Investigations' research trawler *Adolf Jensen* in the dard fashion but the contents of each haul were emptied into a piece of cloth and taken entire, without sieving, to the laboratory. The cold air, freezing point, kept the animals fresh and there was no sign that small chaetes, etc. escaped through the meshes of the cloth. In this way it was ible to take up to 21 hauls on any one day, and to examine them within to three days.

The hauls were taken from sand bottom off the mouth of a small stream ning into the sea between the mountains, Lille Malene and Store Malene . 5). The depths ranged from 8 to 18 metres at various stages of the tide plitude about 3 metres) and can be taken as approximately 10 metres. The , which was coarse with many small pebbles, was mixed with varying unts of alga.

isko Bugt

Six series of hauls were made at four different depths in various localities isko Bugt (Fig. 6; Table 1) between 15 June and 16 July 1956. The hauls e made from the Arctic Station's motor boat *Holk*[1] in the same way as at

[1] Formerly *Holck*.

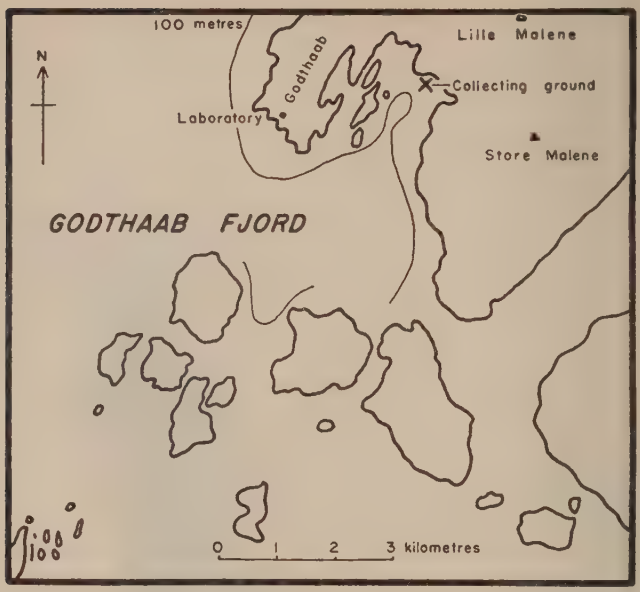


Fig. 5. Collecting ground in Godtha Fjord near Godtha

Godthaab. An additional five hauls (Series 7), taken from the prawn groun in Disko Bugt at 400 metres depth, were also examined.

d. Foxe Basin

From 25 September 1955 to 4 September 1956, Dr. E. H. Grainger, wor ing from the *Calanus*, collected benthos at approximately monthly interv from two stations near Igloolik (Fig. 2). Thirteen large hauls at Station 7 in the bay by the settlement were from thick grey mud at 22 metres depth a four hauls at Station 719 were from mud at about 50 metres in the mouth the same bay.

In 1957 grab and dredge hauls were made at Frustration Bay, Rowl Island (Fig. 2). They consisted of 36 hauls with a Van Veen grab scatter across the mouth of the bay between 3 and 30 metres depth, and several dred hauls down to 50 metres depth. The shores of Frustration Bay consist of coar gravel and stones, replaced below low-water level by finer sediments. Sa bottom was found in several places at about 5 metres depth; and from 10 to metres the sand or stony bottom changed into hard grey, often stagnant, m with a softer superficial brown layer. There were some patches of kelp a other alga at all depths.

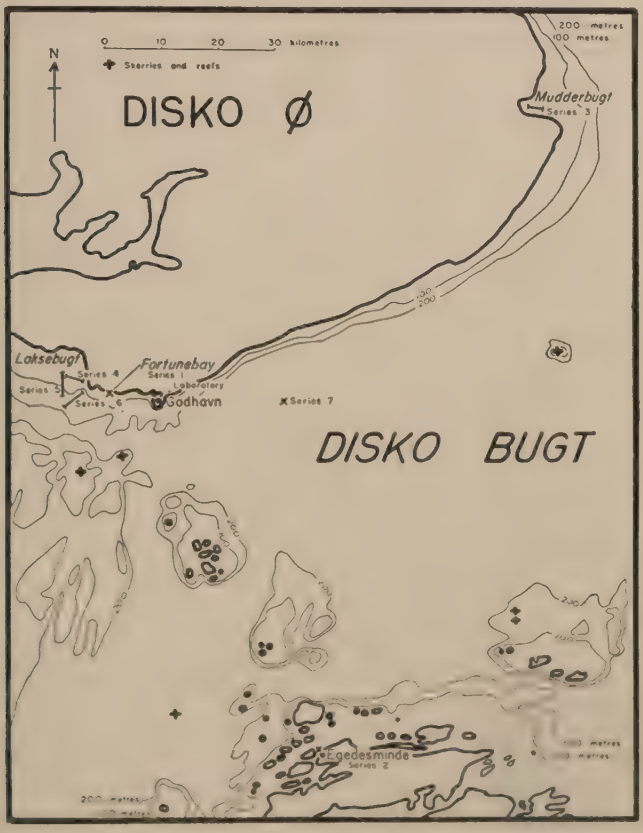
3. Dry organic matter determinations

Species collected in large numbers at Godthaab and Godhavn were deh drated and combusted to determine coefficients to express standing crops terms of dry organic matter as well as of fresh weights of animals. Specime were first dehydrated at temperatures between 100°C and 110°C until t weight became constant; this process took about 24 hours. Shells and tub were then removed from large lamellibranchs and *Cistenides* spp. and these a

nens without calcareous shells were combusted at 1,000°C. Small speci- with shells and tubes which could not be easily removed were combusted 0°C. Combustion was continued until the weight of residue became ant, the process also normally taking about 24 hours. In all cases the weights of specimens dehydrated were greater than 1 gm., but the de- usted residues were weighed to 0.01 gm. The percentages of ash and veights are given to the nearest 1 per cent.

At a temperature of 500°C calcium carbonate does not decompose; its l pressure is slightly greater than 0.1 mm. of mercury at this temperature. ditional precaution was taken by combusting a known weight (4 gm.) of drated calcium carbonate for 24 hours at 500°C. Any loss in weight was an 0.01 gm. Consequently the loss in weight of specimens with calcareous combusted at 500°C does not include losses due to the decomposition of im carbonate.

At 500°C combustion is not quite complete. The difference in the per- ge of the fresh weight left as residue after combustion at 500°C and at °C was generally less than 1 per cent of the fresh weight and hence does eriously affect the accuracy of the results. Any residue left after com- on at 1,000°C for 24 hours is, for all practical purposes, non-combustible.



6. Collecting nds in Disko .

III. Results

1. Composition of the infauna

The species collected, the percentages of hauls in which they occu and their mean numbers and weights per sq. m. are listed in Tables 2–5 fo areas investigated quantitatively. From the data it can be seen that a nu of species numerically dominate the faunas. Now dominance is a rather v concept. It has the general implication that some animals in a fauna are noticeable than others, either through their abundance or through their logical relationships with the rest of the animals. Unfortunately there a hard and fast criteria for selecting dominants, because their noticeability de on so many properties, including for example whether it is a poor or rich which is being investigated. Animals have been regarded as dominant he (1) they occurred in 50 per cent or more of the hauls from an area and h were widely spread, (2) they were markedly more dense than the great ma of species present, or, (3) they represented about 10 per cent or more o total standing crop (fresh weight). Dominance as used here is simp numerical expression and has no biological significance. In Tables 2–5 dom species are starred.

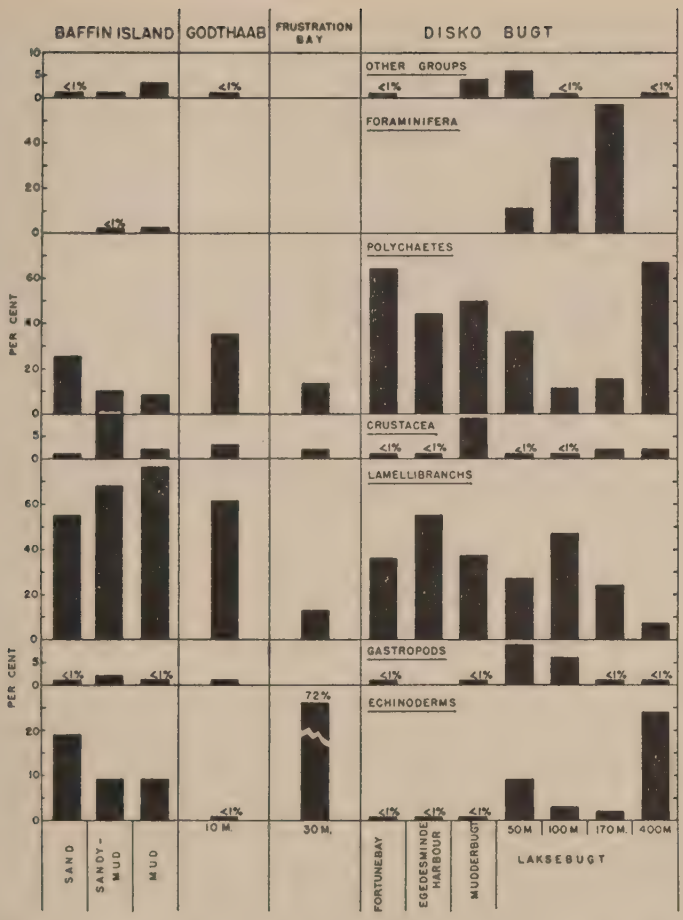
The dominant animals from one locality will be quoted as an exa On mud bottom in north Baffin Island seven species, *Myriochele heeri*, *P medes globosus*, *Astarte borealis*, *Astarte montagui*, *Macoma calcarea*, *truncata*, and *Ophiocten sericeum*, plus unidentified foraminifera and seden polychaetes, occurred in 50 per cent or more of the hauls, which indicated they were widely spread on mud throughout the region. Some species taken in large numbers; the mean number of individuals per sq. m. was 1 more for the foraminifera, *Owenia fusiformis*, and *Myriochele heeri*. So these species occurred in large irregularly scattered groups, e.g., 80 foramin were taken in one haul whereas fewer or none occurred in the neighbou hauls. Several lamellibranchs occurred in heavy masses; there were grou abundant *Astarte borealis* and *Macoma calcarea*, and heavy individual *Serripes groenlandicum*, *Cardium ciliatum*, and *Mya truncata*. Torn sip of adult *Mya truncata* indicated that this species burrowed too deeply t collected accurately.

The infauna in the largest dredge hauls from Igloolik is summarize Table 6, which shows the percentage of hauls in which each species occu Dominant species are starred.

Different species dominate in the different regions investigated, yet s of the faunas are markedly more similar than are others. The significanc these similarities and differences is considered in the Discussion.

2. Standing crops of the infauna

The concept of standing crop provides a means of expressing the am of animals or animal tissue per unit area at any one time. In these surveys numbers and weights of animals per sq. m. in Tables 2–5 are estimates of st ing crops. They have a range from very small, e.g., only 31 gm. per sq between 0 and 3 metres in north Baffin Island to quite high, e.g., 438 gm. sq. m. on sandy-mud bottom between 5 and 47 metres in the same region.



ig. 7.
Percentages of
the mean weights
of animals per sq.
m. contributed
by various
taxonomic
groups in the
regions
investigated.

The percentages of the standing crops represented by major taxonomic groups is shown in Fig. 7. Various groups are dominant in various places. Thus lamellibranchs represent between 60 per cent and 80 per cent of the crop in the areas investigated in north Baffin Island and near Godthaab, but are partially replaced by polychaetes in shallow water in Disko Bugt. Lamellibranchs are an insignificant part of the crop in the deepest Disko Bugt collections, where either foraminifera or polychaetes dominate. In Frustration Bay echinoderms represented 72 per cent of the crop.

Standing crops in Tables 2–5 are expressed as fresh weights, which include water, shells, tubes, organic matter, and non-combustible residues. If the crops are converted into dry organic matter, a much clearer indication is obtained of the mass of animal tissue present on the bottom at any one time. The technique for measuring dry organic matter was described on pages 10–11.

Table 7 shows for several species the percentages of the fresh weights represented by water and dehydrated residue. This residue comprises dry organic matter, ash, and sometimes shells or tubes. The percentage of the fresh

weight represented by these last constituents is shown for some of the specie Since these determinations were made, Thorson (1957) has summarized prev ously published results.

The mean percentage of dry organic matter in species without shells an in species with shells removed is 20 per cent. The values vary between 1 per cent and 25 per cent for different species and for individuals of any or species up to 6 per cent (15 to 21 per cent for *Mya truncata*). In most cas the percentage of ash is small (2 to 4 per cent), but may be as high as 16 per ce in *Cistenides* spp. Thus the residue after dehydration in most species is almo entirely dry organic matter, if shells and tubes are not taken into accoun However, the content of dry organic matter in animals with shells and tub when these are considered in the calculations is much smaller; and varies fro 1 per cent to 17 per cent for different species. Separate values for individua of the same species vary up to 2 per cent (12 to 14 per cent for *Mya truncata*

When converting the standing crop of animals into terms of dry organi matter, allowance must be made for species and individual variation. Th following conversion factors, expressed to the nearest 5 per cent with th exception of the foraminifera, can be applied to the material in the survey described here:

Species	*Conversion factor*
Foraminifera	1%
Errant polychaetes (and shell-less animals)	20%
Cistenides spp.	10%
Sedentary polychaetes (with tubes)	5%
Crustacea	15%
Molluscs (with shells)	10%

A conversion factor was not obtained for infaunal echinoderms. Data i Thorson (1957) suggests 5 per cent as the most appropriate value.

Table 8 shows crops converted into dry organic matter. The ratio dr weight:fresh weight is approximately 1:10 for the shallow-water faunas i north Baffin Island, Godthaab Fjord, and Disko Bugt. This ratio reflects th dominance of lamellibranchs, approximately 10 per cent of whose body weigh consists of dry organic matter. In increasingly deeper water in Disko Bug the ratio becomes greater as the proportion of the total weight represented b foraminifera increases (only 1 per cent of the fresh weight of foraminifera i dry organic matter). The ratio is lowest for the community on the praw grounds in Disko Bugt, which is dominated by non-tubicolous sedentary poly chaetes, 20 per cent of whose body weight is dry organic matter. The rati was high at 30 metres in Frustration Bay where much of the crop consists o large holothurians with a small content of dry organic matter (5 per cent).

The weight of dry organic matter per unit area in shallow water is gener ally 15 gm. per sq. m. or more. The one exception is in Mudderbugt (5 gm per sq. m.), where the weight has probably been badly underestimated becaus of a hard sand bottom. The weight of dry organic matter at depths greate than 50 metres is generally smaller than in shallow water, i.e., about 10 gm per sq. m. or less. There appears to be a difference in the amount of dr organic matter per unit area in the two deep-water communities in Disko Bugt the weights in the foraminifera community between 30 and 200 metres dept were between 2 and 8 gm. per sq. m. and in the deeper water community at 40 metres depth was about 13 gm. per sq. m.

IV. Discussion

1. Quantitative benthos surveys

Most of the benthos collected in arctic North America has been obtained with scraper dredges and trawls. These instruments collect deeply burrowing or active animals very badly. They also bounce over the bottom sampling an unknown surface area. Thus they provide poor quantitative data. Benthic stocks can best be estimated at present by combining scraper dredges, underwater cameras, and grabs or other penetrating samplers (McIntyre, 1956). In the surveys described here, crop estimates are based on grab collections, the most quantitative instrument which could profitably be used under arctic winter conditions. However, scraper dredges were used occasionally, particularly for collecting epifauna.

Effect of variable grab penetration

Grabs are liable to penetrate variable depths into the sea bottom, depending on the experience of the operators and the hardness of the sediment. As a result they give poor quantitative data for deeply burrowing species. (Inevitably, they also give poor data on active and rare species). Nipped-off siphons of *Mya truncata* occurred in some of the hauls from Baffin Island. Large individuals of this species evidently burrowed beyond the depths reached by the grab. As a result the standing crop of *Mya truncata*, and total standing crops wherever this species occurred, may have been badly underestimated.

In order to check the extent of variation introduced into the quantitative data by variable grab penetration the volume of substrate in all hauls was measured during the Godthaab survey. Thirty-two hauls containing much alga were small (mean volume was 2.8 litres), whereas 24 hauls free of alga were generally larger (mean volume was 3.5 litres). Although the difference was not statistically significant ($P>0.05$), the large amounts of alga in the smaller hauls suggested that the grab merely scraped the surface of the bottom when much alga was present but dug deeper where the bottom was free of alga.

The effect of depth of bite of the grab on the collections is illustrated in Fig. 8. There is a steady rise in the mean number of animals per haul up to 3 litres of substrate. Approximately similar numbers of animals were taken in hauls containing between 3 and 5 litres of substrate; the higher numbers in 6 litres volume are based on only 3 hauls, so cannot be taken as evidence for a continued rise. It appears that when the grab merely scraped the surface of the bottom it collected the infauna very poorly, whereas when it penetrated sufficiently deeply to collect about 3 litres of substrate it collected most of the infauna.

In contrast, the weight of animals per haul increased almost regularly with increasing volume of substrate (Fig. 8). As the numbers of animals remained approximately constant above 3 litres volume, the increased weight in hauls of 4 to 6 litres must be due to the presence of heavier individuals in the largest hauls. These hauls contained heavy individuals of *Cistenides* spp., *Macoma calcarea*, and *Mya truncata*, and large numbers of *Ophelia* sp. and *Travisia forbesii*. These species, especially the largest individuals, probably burrow deeply; they will be collected adequately only when a deep-digging grab, which collects large volumes of substrate, is used. Large individuals are usually rare

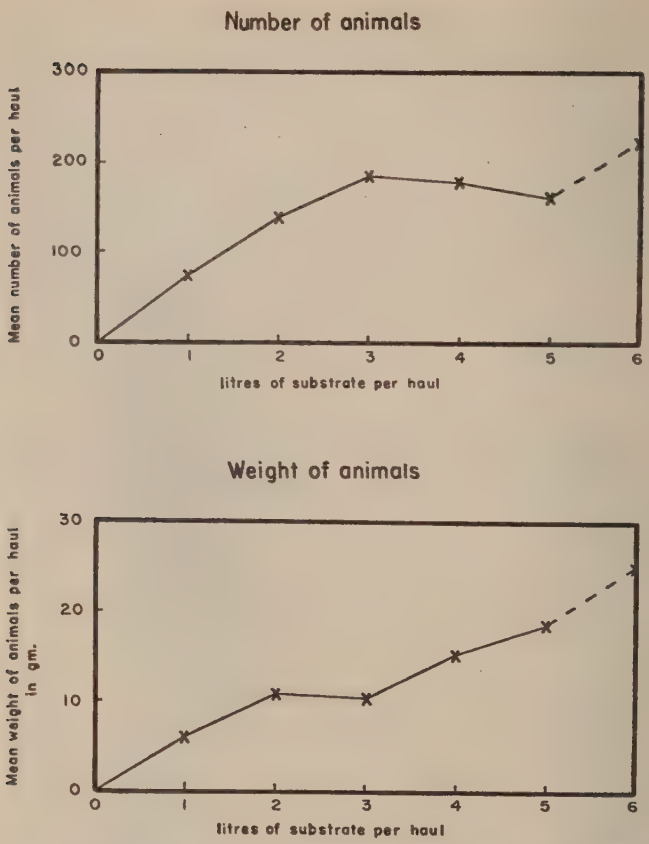


Fig. The effect varying gr penetratic (measured t litres of substra per haul) on t numbers ar weights of anima collected in t Godthaab surve

in arctic regions, hence missing them through poor collecting will have litt effect on density estimates, but may cause serious underestimation of the stanc ing crop by weight.

b. Significance of quantitative data

The first analysis of numerical data from Baffin Island showed that numbe and weights of animals per sq. m. had large variances, and that mean values ha large standard errors and confidence limits. The numerical data were there fore not as reliable as was desired, and were usable only as rough guides t standing crops and relative abundance of different species. In the Greenlan surveys attempts were made to check the reliability of quantitative data obtair able with Van Veen and similar coarse samplers.

Great variation in numbers and weights of animals per grab haul can b due either to biological phenomena or to poor collecting. The Godthaa survey was made to provide some evidence about the relative importance c these two sources of variability. Hauls were made on seven different da from one locality within a total time of one month. The percentage c hauls in which each species occurred often varied in the seven series as muc as 40 per cent but rarely more than 50 per cent (Table 9). Mean numbers an

ghts were also variable; the more abundant species tended to have the atest variation.

Poor collecting can be responsible for some of this variability. Many of extreme values occurred in series 1, 3, and 4, which are based on the smallest nber of hauls: 8, 10, and 7 respectively. The greatest values for some species urred in series 7; the final series when large volumes were being collected. e grab was probably being operated more efficiently by the seventh day's lecting than earlier.

Biological phenomena can also account for some of the variation. Local ironmental differences within the investigated region may cause the fauna vary (Sanders, 1956). The great size range of some species will affect the iability of their weights per haul. Many benthic species are dispersed over bottom in groups (MacGinitie, 1939). The variance in numbers per haul grouped (aggregated) species tends to be large (greater than the mean), of domly dispersed species smaller (equal to the mean), and of evenly dispersed cies smallest (less than the mean).

The type of dispersion can be shown in several ways, of which the cocient of dispersion:

$$\frac{\Sigma (x-\bar{x})^2}{\bar{x}(n-1)}$$

nost convenient for the present material (Clark and Milne, 1955). In this mula x represents the number of individuals per haul, $\bar{x}$ the mean number individuals per haul, and n the number of hauls. The coefficient tends to ty for random dispersion, to less than unity for even dispersion, and to more n unity for aggregated dispersion. Fig. 9 shows that the range of the cocient for species collected in north Baffin Island was generally greater than ty indicating widespread aggregation.

Theoretically it is possible to test the evidence for aggregation from the ues of the coefficient, but as some of the variation in numbers per haul comes m collecting errors, the test would be meaningless with this material.

Where benthic animals are aggregated, quantitative data obtained with b samplers of the Petersen-Van Veen type will inevitably have high varies, and they cannot be as reliable as could be wished even if a very large nber of hauls is obtained. For instance, it will usually be impossible to use istical methods for comparing differences between means.

What conclusions then can be based on these surveys? And how many uls are required?

Collections made with these samplers show which species occur in a large centage of the hauls and hence are scattered widely over the bottom, which cies are numerous in the hauls and hence are scattered densely over the tom, and which species occur as heavy individuals or in heavy groups and nce contribute much to the total weight of animals on the bottom. This ust the type of information which is summarized by the concept of marine el-bottom communities (see page 19).

There is a method of checking if the number of hauls obtained from a gion has been sufficient to collect all the widely spread species. The curves Fig. 10 have been obtained by random selection of hauls from the three ostrates in north Baffin Island. After 15 hauls the rate at which new species cur on mud and sandy-mud drops considerably. On these substrates at least

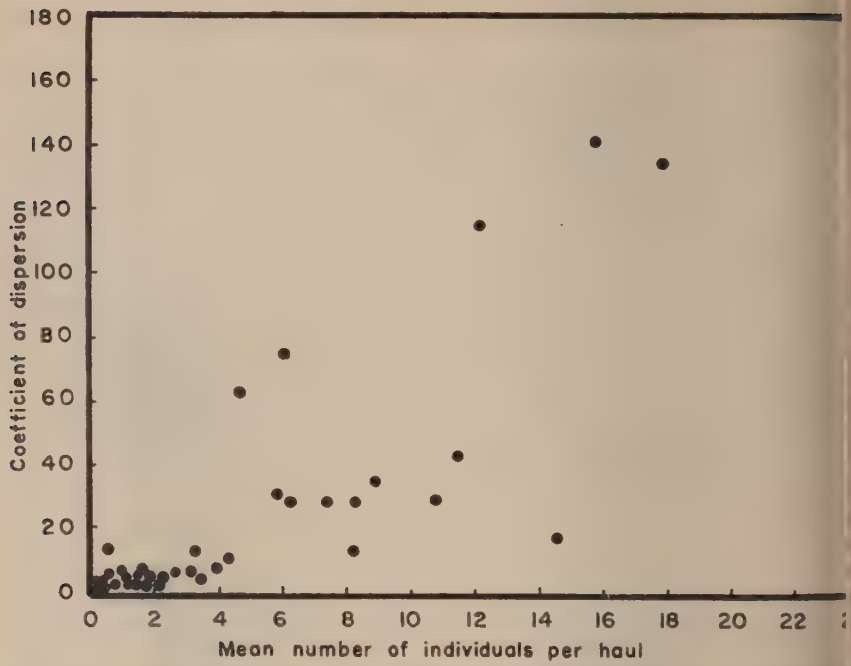


Fig. 9. The relationship between the coefficients of dispersion and the mean numbers individuals per haul for some species in north Baffin Island.

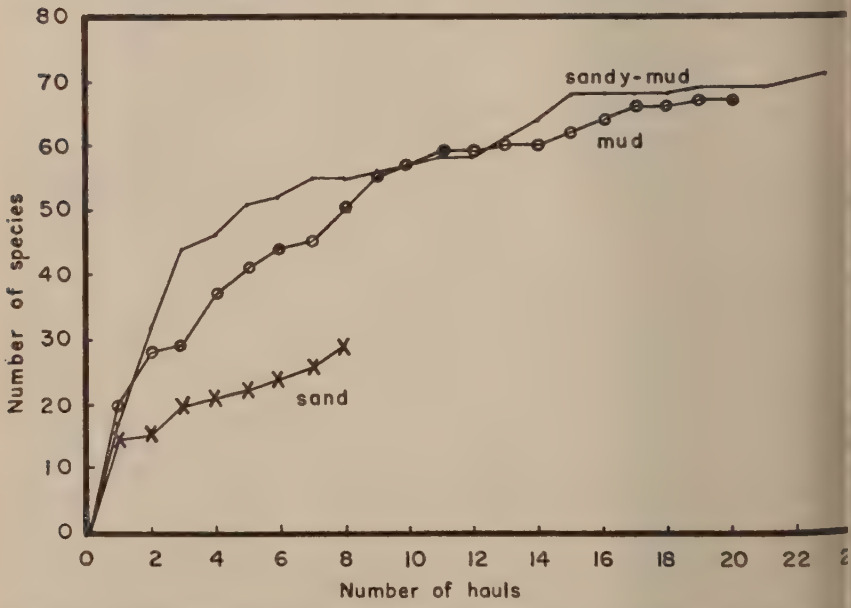


Fig. 10. The relationship between the number of species and the number of hauls fr three substrates in north Baffin Island. At least 15 hauls (of 0.07 sq. m.) were requir before most common species could be collected.

hauls each of 0.07 sq. m. (or a total surface area greater than 1 sq. m.) are uired before all the widely spread species are collected, and the community sent can be adequately determined. This figure of total bottom area to be pled was used as a guide for the later surveys, even though field conditions asionally did not permit keeping to it.

2. Marine level-bottom communities

The results of quantitative benthic surveys show that species associate in h a way that similar faunas are found under similar environmental conditns. The species associations on soft sea bottoms are marine level-bottom mmunities (Thorson, 1957). It is a convention to name these communities er one of the dominant, characteristic species (indicator species). The mmunities are treated here strictly as numerical associations. They may o be ecological associations, but this aspect of their biology has not been died during these investigations.

This concept of communities is particularly useful as a means of summarng the infauna of a region where there are few species, but many of them ndant, some of which have strict environmental requirements and a limited tribution. These criteria apply to the arctic regions. Few infaunal species cur (compared with the tropics for instance), but many of them are abundant, l some occur in easily definable environmental niches.

Undoubtedly many arctic species tolerate a wide range of conditions. *coma calcarea* is one obvious example. There are others, particularly polyaetes, which are so widespread that they occur in several recognized comnities both in the Arctic and in warmer marine regions. This however is evitable when trying to apply a scheme of classification to what are, after almost infinitely variable faunas scattered over vast tracts of level sea ttom with few sharply defined environmental boundaries.

Known arctic communities

Marine level-bottom communities described from the arctic regions include allow-water *Gomphina fluctuosa*, *Portlandia arctica*, and *Macoma* comnities, and deeper-water foraminifera and *Astarte crenata* communities horson, 1957; Ockelmann, 1958). *Macoma* communities may contain a stinct, upper, *Astarte* zone. There is also a little known *Chiridothea sabini* mmunity (Vibe, 1939).

Characteristic dominant species in these communities are listed by Thorson 957), and their principal distinguishing features are summarized in Fig. 11. he model system developed by Danish authors is not used here, as it gives e impression that infaunal animals are scattered uniformly over the bottom, hich they most decidedly are not (MacGinitie, 1955, and see p. 17). The nellibranch constituents of arctic communities have recently been described detail by Ockelmann (1958), who has changed a number of the original mmunity names. These changes have been adopted here, although they ere not used in a previous reference to these investigations (Ellis, 1959).

Communities in Baffin Island, west Greenland, and Foxe Basin

The stars in Table 2 indicate the species which dominate the infauna in allow water in north Baffin Island. They include many of the species charterizing the arctic *Macoma* communities (Fig. 11). Large numbers of

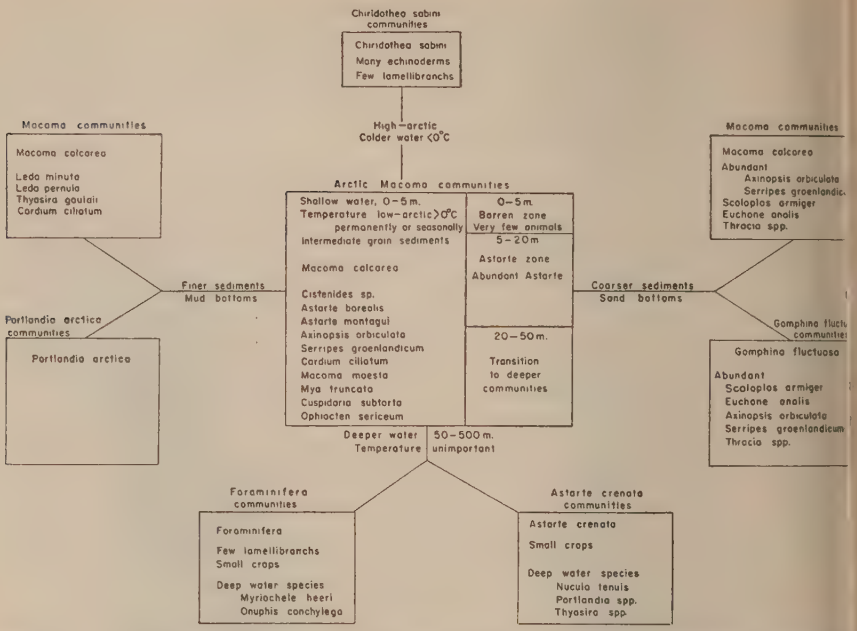


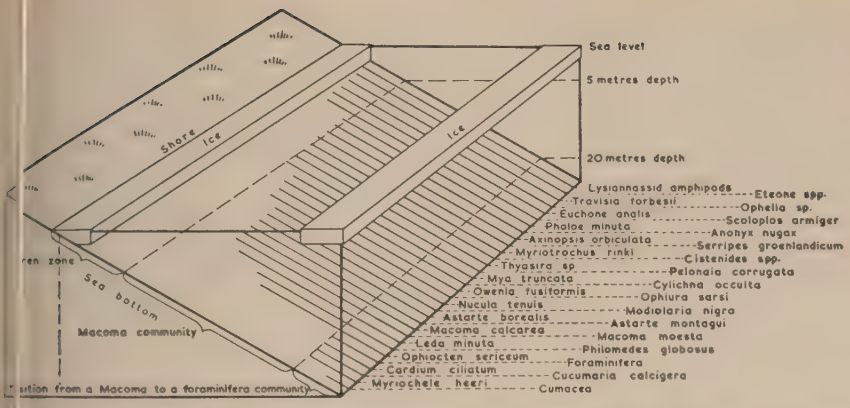
Fig. 11. The principal characteristic features of known arctic level-bottom communiti

Astarte borealis and *Astarte montagui* occurred to depths of about 20 m. an suggested the presence of an upper *Astarte* zone similar to those found in ea Greenland (Thorson, 1933). Below 20 metres to at least 50 metres the faun included an increasing number of species characterizing the foraminifera com munities, and appeared to be in the process of changing from a *Macoma* com munity. These community changes with depth are summarized in Fig. 12.

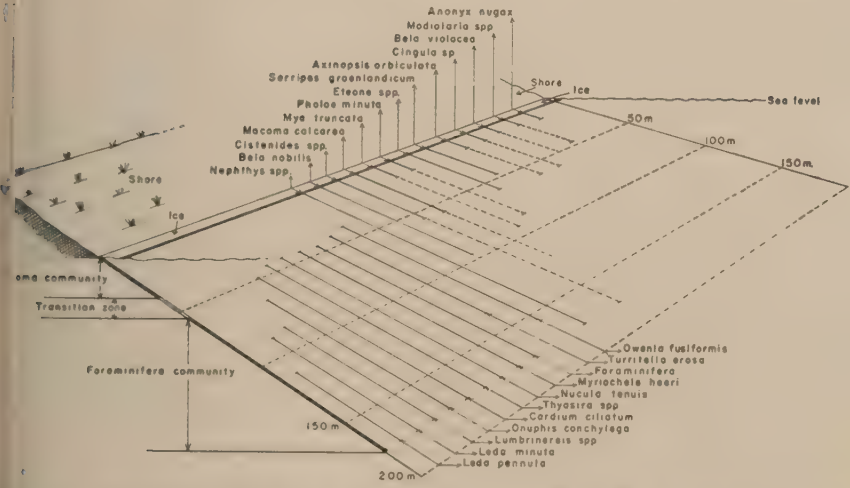
The sandy bank sampled at 10 metres depth near Godthaab (see Table 3 also supported a *Macoma* community, although differing in many details fro the fauna in north Baffin Island.

The shallow bottoms sampled in Disko Bugt (Table 3) all supporte *Macoma* communities, although the faunas in Fortunebay and Mudderbug were more alike than either were to the fauna in Egedesminde harbour. Betwee 30 and 60 metres depth, the dominant animals suggested that the fauna wa changing from a shallow *Macoma* community to a deeper foraminifera com munity. In deeper water still, particularly between 100 and 170 metres, th dominant animals (Table 4) indicated a foraminifera community. The com munity changes with depth are summarized in Fig. 13. At 400 metres th fauna seemed unusually constant in spite of there being only 5 hauls available Table 4 shows that several of the species occurred in 3 out of the 5 hauls. Th small sample and unusual species association prevents applying a communit name at present.

The fauna in Frustration Bay, Rowley Island, comprised principall abundant *Nephthys* spp., and sedentary polychaetes with consistent but smalle numbers of *Chiridothea sabini*, *Macoma* spp., *Yoldia* sp., Ophiuroids, an



12. Depth ranges for the most abundant species in the north Baffin Island collections. I re is a faunal zonation with depth consisting of a progressive change from a barren low zone (0–5 m.), to *Macoma* community (5–20 m.), and to foraminifera community below 20 m.



13. Depth ranges for the most abundant species in the Disko Bugt collections. Infaunal nation with depth consists of shallow-water *Macoma* communities being replaced by foraminifera communities between 30 and 60 m.

Cucumaria calcigera. This faunal association, the unusually high percentage of te crop (by weight) represented by echinoderms (Fig. 7), and the low per-ntage represented by lamellibranchs suggest a *Chiridothea sabini* community nilar to that found at Kap York, west Greenland, by Vibe (1939).

At Station 723 in the bay by the settlement of Igloolik, the infauna on ick mud at a depth of 22 metres contained large numbers of *Portlandia arctica* d *Chiridothea sabini* but few of the species comprising the *Macoma* com-unities. The fauna was closest to those described by Thorson (1934) as the *rtlandia arctica* communities of east Greenland. These communities also

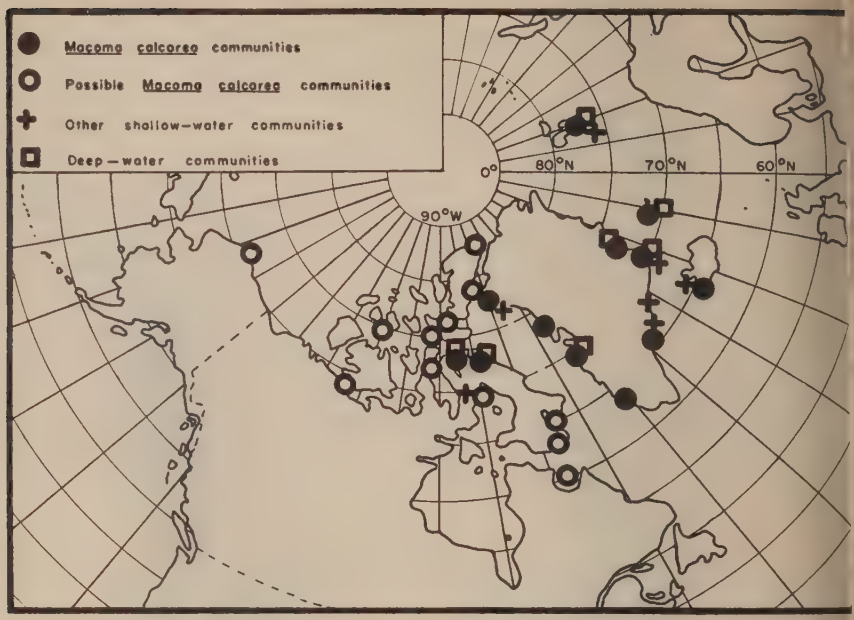


Fig. 14. Distribution of known marine level-bottom communities in arctic north Americ

occur near Spitsbergen according to Thorson, and the material from Iglooli now suggests that they have wider distribution than previously realized.

At Station 719 near Igloolik on mud at 50 metres depth some of the *Macom* community indicator species were present but there were also abundan *Chiridothea sabini*, *Yoldia* sp., *Portlandia intermedia*, and *Ophiocten sericeum* This association of animals suggests that the fauna is in the transition zon between shallow- and deep-water communities, but that the shallow-wate community may not be a typical *Macoma* community. The *Chiridothea sabin* in the fauna suggests it may be similar to that described from Kap York.

c. Distribution of communities in arctic North America

Macoma communities have now been found widely in arctic North Americ (Fig. 14). In addition published reports of dredge collections contain many of the arctic *Macoma* communities' indicator species. They have been taken from north and south Ellesmere Island, Cumberland Sound, Frobisher Bay Ungava Bay, Foxe Basin, Lancaster Sound, Melville Island, Port Kennedy Dolphin and Union Strait, and Point Barrow (Figs. 1 and 2). A list of abou 70 references describing these collections is given by Ellis (unpublished thesis) The widespread distribution of these indicator species suggests that *Macom* communities are also widely spread in arctic North America. They seem t be the commonest communities in shallow arctic water being displaced by others only under localized, special conditions.

Quantitative deep-water collections between 50 and 500 metres depth i arctic North America have resulted in descriptions of two faunal associations the *Astarte crenata* and foraminifera communities. Most non-quantitative

ections in arctic Canada and Alaska do not extend to sufficient depths to onstrate changes in the composition of the infauna with increasing depth. wever there is some evidence available. Dredge hauls near Igloolik est that a change in the fauna occurred at about 50 metres depth. Dredge ections from below 50 metres in Ellesmere Island, Dolphin and Union Strait, Point Barrow (for reference see Ellis, unpublished thesis) all include some he deep-water communities' indicator species. Bottom samples taken een Baffin Bay and Melville Island in 1947 at depths from 30 to 800 metres erally contained large numbers of foraminifera (Phleger, 1952). The lts of the few, widely scattered deep-water collections of benthos in arctic th America all suggest that there is a change in the composition and abunce of the infauna between 20 and 50 metres depth; and that in many places change is from a *Macoma* to a foraminifera community.

3. Factors affecting the composition and standing crop of arctic infaunal benthos

eriodic population fluctuations

The infauna present in any locality may show considerable fluctuations n season to season and from year to year (Jensen, 1919; Blegvad, 1928 and 1). Fluctuations of this type occur where many of the constituent species short-lived and reproduce by means of pelagic larvae (Thorson, 1950). ther of these conditions apply to the arctic infauna, since 95 per cent of the minating species, representing all taxonomic groups, reproduce without a agic larval phase and the heavy abundant lamellibranchs tend to be long- ng forms (Thorson, 1936). Consequently, the arctic infauna does not have eavy mortality at the end of an annual breeding season with poor replace- nt due to a reduced spatfall. Furthermore, young individuals are apparently exposed to a high rate of predation because predatory animals are only nor constituents of the benthos (Thorson, 1957) and so the rate of turnover robably small. Consequently it is unlikely that periodic population fluctu- ns cause great changes in the composition of the arctic communities, hough they will undoubtedly affect the abundance of the few species such *Mya truncata* which do reproduce with pelagic larvae. Therefore, quan- tive surveys made at any one time should give a satisfactory description of tic communities.

Fluctuating environmental conditions

In regions where the environment fluctuates, one series of collections may nt adequately describe the communities as they exist throughout the whole ssible range of conditions. Benthos in a region with changing or fluctuating eanographic conditions tends to be a mixture of the species characterizing th preceding and existing conditions, unless there has been a catastrophic ange. This applies especially to the arctic regions where many species are g-lived and reproduce non-pelagically.

The epifauna of southwest Greenland is an excellent example because the pically arctic species' associations include some animals which do not occu purely polar water, for example *Pandalus borealis* (Horsted and Smidt, 1956). eir presence in southwest Greenland reflects the influence of warm Atlantic

water from the south. Occasionally cold polar water appears along this co and has a catastrophic effect on the stock of *Pandalus borealis*, and presumab on other species with similar distribution. Benthic surveys in such regio may require more extended collecting than in more stable areas.

c. Substrate

Thorson (1957) has reviewed the relationships between marine lev bottom communities and substrates. In the Arctic, for instance, *Portlan arctica* communities have been found only on soft mud, especially off glacie and river mouths, and *Gomphina fluctuosa* communities only on sand botto Arctic *Macoma* communities, however, tolerate a wide range of substrates, a this is reflected by their variable composition.

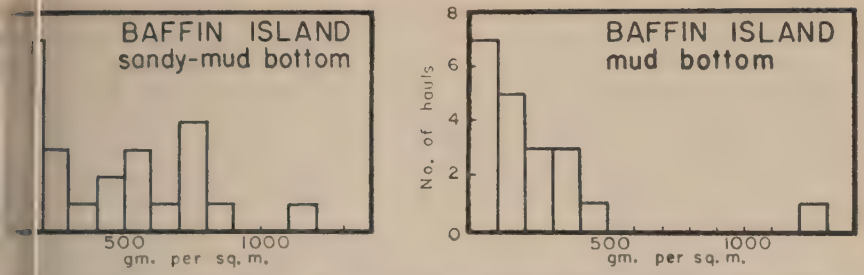
Thus in north Baffin Island many species occurred on part only of the tot range of substrates sampled. For instance, sand bottom or sand and sandy-mu together, supported more *Eteone* spp., *Travisia forbesii*, *Ophelia* sp., *Euchon analis*, lysiannassid and oedicerotid amphipods, *Serripes groenlandicum*, *Cylichn occulta*, *Bela violacea*, and *Myriotrochus rinki* than did mud. On mud botto or mud and sandy-mud together, there were more foraminifera, *Myrioche heeri*, *Philomedes globosus*, and *Ophiocten sericeum* than on sand, althoug this may have been a depth effect as mud tended to occur at greater dept than sand. A few species were markedly more abundant on sandy-mud tha on either mud or sand: *Pholoe minuta*, *Cistenides* spp., *Astarte borealis*, *Astart montagui*, *Macoma moesta*, and *Cucumaria calcigera*.

The Godthaab *Macoma* community included a number of sand-botto animals not collected in north Baffin Island, e.g., *Nebalia bipes* and *Diapha minuta*.

The sand bottoms sampled in Fortunebay and Mudderbugt supporte similar faunas, which were quite different from the mud bottom fauna i Egedesminde harbour, although all three were within the limits of variabilit of arctic *Macoma* communities. For instance large numbers of *Pholoe minut* and *Axinopsis orbiculata* were taken from the two sandy areas, but few or non from mud.

Infaunal standing crop also appears to be influenced by substrate. I north Baffin Island (Table 2) faunal densities on sand and mud below the barre uppermost 5 metres were similar (1,082 and 987 animals per sq. m. respectively) but were noticeably higher on sandy-mud (1,815 animals per sq. m.). Cro weights were also similar on sand and mud at these depths (201 and 200 gm. pe sq. m. respectively) and higher on sandy-mud (438 gm. per sq. m.). Frequency diagrams of animal weights per sq. m., based on individual grab hauls, also suggest that the crop of animals on sandy-mud is greater than on mud (Fig. 15) The distributions show left-hand skew due to the predominance of small hauls On mud bottom 7 hauls give weights less than 100 gm. per sq. m. and 13 greater whereas on sandy-mud all hauls give a calculated weight per sq. m. greate than 100 gm.

In the Godthaab survey (Table 3) the calculated weight of animals pe sq. m. was 133 gm. and was therefore lower than the value on sand ground i north Baffin Island. This could have resulted from poor penetration by the grab, as many large, torn specimens of *Mya truncata* were collected. The actual weight of animals is probably larger than this survey shows. The density



15. The relative crop sizes on sandy-mud and mud bottom in north Baffin Island as shown by frequency distributions of weights of infauna per sq. m.

nimals in the Godthaab survey is as high as in Baffin Island, due principally warms of juvenile lamellibranchs.

In Disko Bugt (Table 3) the density of animals was as high in Mudderbugt 107 per sq. m.) and in Fortunebay (1,528 per sq. m.) as in north Baffin id, but few animals were collected in Egedesminde harbour (432 per sq. m.). weight of infauna per sq. m. in Fortunebay (296 gm. per sq. m.) and desminde harbour (320 gm. per sq. m.) was comparable to those in north in Island, but was much smaller in Mudderbugt (48 gm. per sq. m.). These data reflect population differences. Mudderbugt fauna consisted almost rely of small species and juveniles of large species; but as the grab pene- d the substrate badly it may have missed heavier individuals of the larger ies. Mudderbugt's low faunal weight could also have resulted from lethal cts of runoff from a nearby river. The low density of animals in Egedes- de harbour was probably due to the foul bottom limiting the variety and ndance of species.

The dredge hauls from Igloolik do not permit any conclusions on the size he crop there.

In Frustration Bay (Table 5) the shores were generally barren. On sand om at about 5 metres depth there was a scant fauna with a crop of 35 gm. sq. m. At 15 metres depth, where sand had been replaced by finer sedi- nts, the crop was larger (210 gm. per sq. m.) due to many big *Serripes enlandicum*. A patch of soft stagnant mud at the same depth (not referred n Table 5) had a very low crop (only 8 gm. per sq. m.), and as the grab ected well from this bottom the low value was undoubtedly real. At 20 res depth the bottom consisted of mud and stones and the grab collected ly so that the low value (93 gm. per sq. m.) could be a bad underestimate. 30 metres the crop was again high (290 gm. per sq. m.) with heavy *Nephthys* ., *Yoldia* sp., *Serripes groenlandicum*, *Cucumaria calcigera*, and asteroids.

The crops between 200 and 300 gm. per sq. m. are close to those generally nd in arctic North America at depths between 5 and 50 metres. The smaller ps at 5 and 15 metres appear to be real phenomena arising from ice scouring e page 29) and stagnant bottom respectively, whereas the smaller crop at metres depth is probably not real, but an underestimate due to poor grab etration.

There is another possible cause of low crops related to substrate. This been referred to by Thorson (1934), who deduced that low crops in *coma* communities at the heads of east Greenland fiords was due to poor

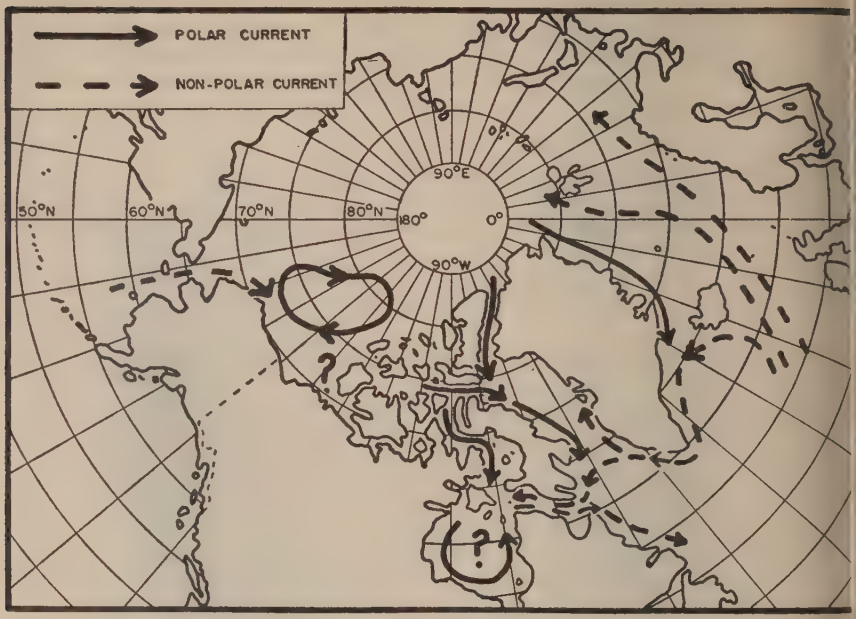


Fig. 16. Arctic North America showing polar currents and the areas of mixing wit non-polar currents.

supply of nutrient organic matter. Thorson maintains that communities situ ated near the open sea benefit from a greater nutrient supply than those in fiord stretching a hundred kilometres or more inland.

Good nutrient supply is a possible explanation of the high crops on sandy mud bottom in north Baffin Island. Sandy-mud bottoms sampled were clos to the mouths of rivers and creeks, which provide nutrient material to the se (Sverdrup, Johnson, and Fleming, 1942), and the same authors present evidenc that fluvial deposits around river mouths have a locally high organic conten Sand bottoms sampled were also near rivers but their crops were low. Thes were all in shallow water exposed to limiting effects of ice scouring and mel water (see page 29), and their productive potential would therefore not b realized.

The effect of a change in substrate on the fauna has been followed b MacGinitie (1955) at Point Barrow, Alaska. He described how the offshor fauna changed considerably during the winter of 1948–9, when river mud wa deposited over a large area.

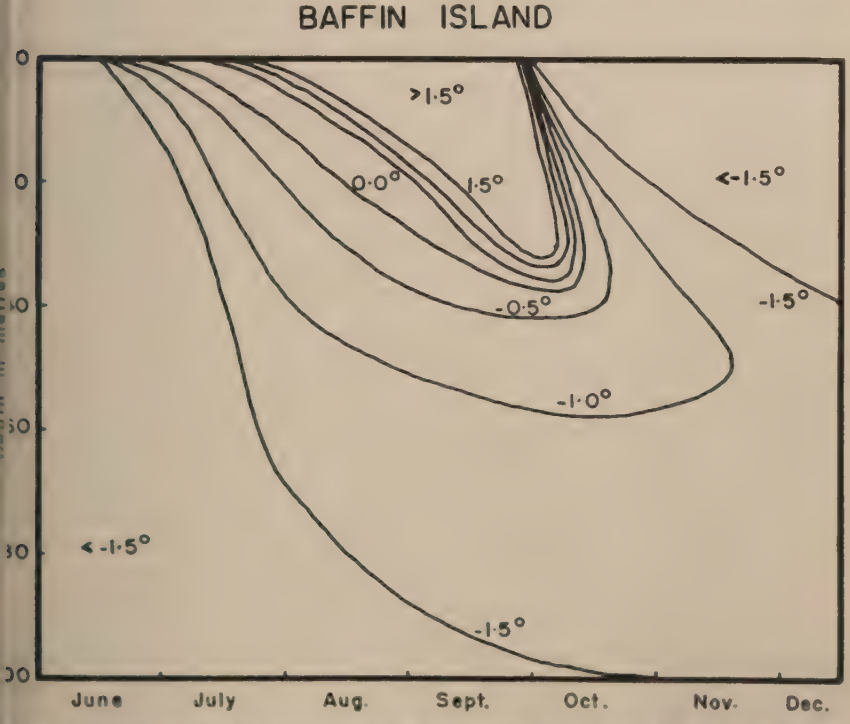
d. Oceanography

The upper 200–300 metres of water throughout most of arctic Nort America originates in the Polar Basin at the same depth. This water characte istically has negative temperatures and a salinity about 33‰ or less (Dunba 1951; Bailey, 1955 and 1957). The distribution of such water flowing directl from the Polar Basin is shown in Fig. 16. The characteristics of deeper wate are described by Bailey (1957).

The high latitudes and presence of ice in these regions ensures that the hotic zone is small. The depth from which phytoplankton can be collected ndantly indicates empirically that this zone reaches a maximum depth of ween 30 and 50 metres. Within the zone the water warms in summer ally, but not always, to positive temperatures and cools in winter almost to zing point (Digby, 1953; Ellis, 1956; Bailey, 1957; Grainger, 1959). Regions ere summer warming produces positive temperatures are often called "low-tic" (Ekman, 1953), whereas regions where temperatures remain negative ughout the year are referred to as "high-arctic". These terms "low-arctic" "high-arctic" can also be applied to vertically distributed zones in the sea ause "low-arctic" temperatures overlie "high-arctic" temperatures in many alities during the summer months (Fig. 17).

In some far north regions summer warming of sea water is negligible and s for only a few weeks, as in the vicinity of the northern Ellesmere ice shelf ary, 1956) and Frustration Bay (unpublished data from the *Calanus* expedi-n, 1957). High-arctic temperature conditions may be almost permanent in region where fast or drift ice usually remains throughout the summer.

In the greater part of arctic North America summer insolation is more longed. It often causes a stable, brackish layer to form at the surface, the ord-water", with temperatures occasionally greater than 5°C and salinities



. 17. Seasonal changes of temperature with depth off north Baffin Island to show the ects of summer insolation in polar water. Positive temperatures may overlie negative temperatures. (Data from Ellis, 1956).

possibly near zero (Thorson, 1934). This layer is very variable and ma extend to 5 or more metres depth. Insolation is also often sufficient for positiv (low-arctic) temperatures to reach depths of 20 to 50 metres before approach ing winter brings about a general surface cooling in September or Octobe (Fig. 17). Below the surface-warmed water, the rate at which temperatur decreases with increasing depth is occasionally so great in late summer that i suggests the presence of a thermocline coincident with the lower level of th euphotic zone. Such a thermocline may not be normal. It may develop onl if there is unusually steady insolation during the summer. Nevertheless a rapi drop in temperature can be expected most years (below the insolated layer at depths between 20 and 50 metres.

Throughout arctic North America the sea surface begins to cool toward the end of August and during September, and the summer stratification the breaks down. Surface ice starts to form in September or October. Wate between 50 and 100 metres depth, which often warms slightly during th summer, may not return to its winter temperature until December or late (Fig. 17). Data so far available for the winter months indicate that fron December to May polar water is thoroughly mixed without sign of stratification

Apart from temperature and salinity cycles there are other changes in th water of the euphotic zone, the sum of which results in a marked seasona upgrowth of plankton and a reproductive outburst by some of the mor abundant shallow-water benthos (Thorson, 1957; Ockelmann, 1958). Th length of the productive season is variable both from place to place and fron year to year; it may last barely a few weeks (Appollonio, 1957; Grainger, 1959) or several months (Digby, 1953).

In southern parts of arctic North America, polar currents mix with warme and more saline water from the Atlantic and Pacific oceans (Fig. 16). This i so near Point Barrow (Johnson, 1956), Hudson Bay (Bailey and Hachey, 1951a) in Hudson Strait, southern Baffin Island, and Ungava Bay (Dunbar, 1958), the Labrador coast and southwest Greenland (Hachey, Hermann, and Bailey, 1954) and southeast Greenland (Hermann, 1957). Oceanographic conditions in the eastern Beaufort Sea are not well known but appear to show no non-polar influence (Cameron, 1953).

These regions with water of mixed polar and non-polar origin are known as the subarctic regions (Dunbar, 1951); in them, the seasonal fluctuations near the surface occur over water which tends to be warmer and more saline than purely polar water. Nevertheless the surface conditions down to about 50 metres are essentially the same as the conditions farther north; during winter temperatures are negative, often near freezing (−1.7°C), but summer insolation raises the temperatures and lowers the salinities. In these regions "low-arctic" conditions may occur seasonally at the surface, or be permanent due to the intermixing of non-polar and polar currents.

The oceanography of a region influences the fauna through three dimensions. Consequently a discussion of its effects can be conveniently split into sections concerned with depth (vertical) effects and latitude (horizontal) effects. In these surveys too there are some combined depth and latitudinal effects which are discussed separately.

i. Depth effects: Danish quantitative surveys in Greenland laid the basis for describing arctic infaunal zonation with increasing depth. This zonation has

e major components (Figs. 12 and 13). At the surface, from 0–3 metres, is almost barren layer kept clear of life by ice scouring, freezing, and the porary occurrence of a low salinity "fjord-water" layer during summer horson, 1933). Slightly deeper, from 5–50 metres, is the most densely ulated zone, usually comprising a *Macoma* community. Deeper still, generl below 50 metres to at least 500 metres depth, are the impoverished deeper faunas, so far known as two distinct associations, the foraminifera and *arte crenata* communities. These have been found at all depths between e two levels in polar water, mixed polar and non-polar water, and deep ntic water beneath the polar currents. Evidently they are little affected the oceanographic differences between these characteristic water masses.

There are of course wide areas of gradation between the three zones. as the barren zone gives way to shallow-water communities between 3 and 5 res; *Macoma* communities merge gradually into foraminifera communities ween 20 and 50 metres.

What is the cause of the widespread faunal change between 20 and 50 res depth? It is just at this general level that there is a rapid drop in perature during the summer months as a result of surface insolation (Fig. . Can this be a causal relationship? The biology of some species suggests t it is.

It has long been known that the vertical as well as the geographical range many marine species are limited by sea temperature (Murray and Hjort, 2; Orton, 1920). For instance Lemche (1941a, 1941b) deduced that in the tic regions some opisthobranch molluscs only matured and spawned within ricted temperature ranges. They were apparently bound to the depths at ich temperatures suitable for maturation and spawning occurred (even if y for a short period of the year). Occasionally individuals were found side the normal limits of abundance of the species, but these had presumably tled accidentally at unusual depths and were unable to breed. Some of the sthobranch molluscs reproduced only in shallow water; some only in deeper ter; and some were independent of whatever factor imposed the vertical its on the others.

Lemche's reasoning can be applied to the infaunal benthos in general. ne of the abundant species in shallow water spawn only during the summer horson, 1936) and appear to depend on warm water in order to reproduce. e deeper living species spawn in water almost unaffected by insolation with manently negative temperatures. These species do not depend on the temrary summer conditions near the surface in order to reproduce. Some cies reproduce both in the euphotic zone (some in summer and some in nter) and below; they are adapted to conditions in both shallow and deep lar water.

The *Macoma* and other shallow-water communities in arctic North nerica comprise species reproducing principally in the euphotic zone and ers capable of reproducing both in and below the zone (some of the latter up are, however, most numerous in the euphotic zone). The deeper-water mmunities comprise species reproducing both in and below the euphotic ne and others only able to reproduce below the euphotic zone. The oceanraphic conditions in purely polar water whereby a warm, insolated layer is perimposed in summer on permanently cold water is responsible for the

presence of distinct shallow-water infaunal communities overlying deeper-wate communities.

There are extensive regions in arctic Canada with permanent or almos permanent ice cover (Jenness, 1953; Moira Dunbar, 1954), but, unfortunately almost nothing is presently known about the extent of the euphotic zone an the vertical distribution of the infaunal communities in these regions, apar from the presence of *Chiridothea sabini* communities in the shallow water o Melville Bugt and Frustration Bay. The benthic fauna at Melville Bugt wa unique when described by Vibe (1939) and was ascribed partly to the high arctic marine environment and partly to the nearby presence of a vast breedin colony of dovekies. The similarity with the Foxe Basin fauna now suggest that the marine conditions are most important, because the environments i Frustration Bay and near Igloolik are both high-arctic, but there are no nearb breeding colonies of sea birds (Ellis and Evans, in press).

ii. Latitude effects: Towards the southern limits of arctic North Americ the water becomes warmer and more saline due to the influence of Atlantic o Pacific currents (the subarctic regions); and the marine fauna at all depth includes a gradually increasing number of species which do not occur in pola water, e.g., *Macoma baltica* and *Mya arenaria*. Different species may be asso ciated with either Atlantic or Pacific influences, e.g., *Cardium fasciatum*, *Syn dosmya nitidum*, and *Cyprina islandica* appear in the fauna near the Atlantic Ocean, and *Macoma oneilli* and *Mya intermedia* near the Pacific Ocean. Asso ciated with these faunal changes will be gradual changes in the communities, fo instance, the boreal *Macoma baltica* communities will gradually replace th arctic *Macoma* communities.

Most of the infaunal species taken in these surveys are spread throughou arctic North America and many are circumpolar; but nevertheless some specie are limited to either eastern or western sections. For instance, althoug *Diaphana minuta* occurred in southwest Greenland, it has never been taken in Baffin Island or north of 71°N. in Greenland (Lemche, 1941a). *Cingula* sp. in Table 3 is possibly *Cingula globulus* (see Appendix), which has northern limits similar to those of *Diaphana minuta*, and there are yet other species (which were not taken in the Godthaab survey) with similar northern limits. e.g., *Cyrtodaria kurriana* and *Macoma baltica* (Thorson, 1951). At least two epifaunal species taken in the Godthaab survey, *Hyas araneus* and *Hyas coarctatus*, have the same pattern of distribution. Many Pacific species pene trate only to the north coast of Alaska, although some may reach as far east as Dolphin and Union Strait, and a few to Greenland and Ungava Bay (Dunbar, 1954; Grainger, 1954 and 1955). Many Atlantic species are recorded from southwest Greenland and Hudson Strait, although a few occur in north Baffin Island, and some even at Point Barrow (MacGinitie, 1955; Berkeley and Berkeley, 1956). Some Atlantic or Pacific species can therefore be expected almost anywhere in arctic North America, although the region between Victoria Island and Baffin Island is likely to contain few such species. This region can be regarded as a zoogeographic boundary between Atlantic and Pacific influences on the infaunal benthos of arctic North America.

Some animals are restricted to the northern part of the arctic regions. For instance, *Macoma moesta* has been taken in north Baffin Island but not south west Greenland. There are other species with a similar northerly distribution, e.g., *Portlandia arctica* (Thorson, 1951).

Some infaunal species are therefore limited to only part of arctic North America, and their absence elsewhere appears not to be due to inadequate collecting but to fundamentally different environmental conditions summarized by the terms "arctic" and "subarctic". Nevertheless differences in infaunal composition and crop in arctic and subarctic regions appear to be slight, and essentially similar *Macoma* communities exist on similar substrates under the two sets of oceanographic conditions.

iii. Combined depth and latitude effects: Many of the arctic, shallow-water species, and the communities which they form, penetrate to greater depths in regions where polar water mixes with non-polar water (Thorson, 1941; Ekman, 1953) than in purely polar water. Both Brotsky (1930) and Idel'son (1930) recorded *Macoma* communities at almost 100 metres near Spitsbergen and Parat and Devillers (1936) found the same communities at similar depths near Jan Mayen. MacGinitie's (1955) collections from Point Barrow recorded many species between 100 and 200 metres which have generally been taken only at smaller depths in polar water.

A little can be said about the composition of the deep-water communities in regions with mixed polar and non-polar water. Horsted and Smidt (1956) showed that the various prawn grounds at about 400 metres depth in Disko Bugt and elsewhere in southwest Greenland had different benthonic faunas, explainable in part by the presence of cold or warm water, but actually their species lists suggest the presence on all grounds of essentially similar infaunal communities whose detailed composition only is modified by local temperature differences.

Oceanographic evidence indicates that there are pockets of high-arctic conditions in the low-arctic subregion. The offshore water along the Labrador coast is low-arctic according to data in Bailey and Hachey (1951b), but Nutt and Coachman (1956) show that Hebron Fiord has high-arctic temperatures due to a submarine sill forming a threshold which prevents free entry of water from the Labrador current. However, the surface water in the fiord acquires low-arctic conditions temporarily in summer through insolation. Other Labrador fiords have similar high-arctic conditions in deep water (Nutt, 1953). Kangerdlugssuaq Strømfjord in west Greenland also has high-arctic conditions in deep water (Wesenberg-Lund, 1950a) due to the presence of a sill at its mouth. These pockets of high-arctic conditions can be expected to have a more arctic fauna than the surrounding low-arctic areas and, in fact, Wesenberg-Lund (1950a) and Horsted and Smidt (1956) both show that the benthos in the threshold fiords of southwest Greenland has a more arctic character than that of the open fiords with warmer water.

4. Productivity

The standing crop of benthos expresses the number or weight of animals per unit area of bottom at any one time. The crop is the momentary realization of the opposed processes of production and destruction, consequently the size of the crop gives no direct information about the actual rate of production of animals on the sea bottom. For instance, the weight of the infauna in the arctic regions varies from less than 50 gm. per sq. m. in deep water to almost 500 gm. per sq. m. in shallow water at one locality in west Greenland (Vibe, 1939). This range of values is as great as in warmer regions (cf. Spärck, 1935)

if not greater, but it does not imply as great a rate of benthic production, because the size of the crop tells nothing about the rate at which it is destroyed and replaced.

There are several observations, however, which indicate that biological processes operate at lower rates in the Arctic than in warmer regions, although there is also good evidence for some thermal acclimation of metabolic processes (Dunbar, 1957). The standing crop (by weight) in arctic shallow-water communities is dominated by lamellibranchs which live for several years, whereas in warmer regions the dominant species may be largely the result of one year's production (Jensen, 1919; Smidt, 1951). Arctic species generally reproduce non-pelagically and recruitment of such species tends to be at a more uniform and lower rate than of species which reproduce with pelagic larvae (Thorson, 1946 and 1950). The rate of predation is small in the arctic regions (Thorson, 1957) where the proportion of the benthic crop represented by predatory polychaetes, gastropods, and echinoderms is low. The annual turnover of animals in the arctic shallow-water communities appears to be small and less than in the infaunal communities of warmer regions, where the annual production of benthos may be from 2 to 5 times as great as the standing crop at any one time (Sanders, 1956). In the Arctic the annual production will probably be less than the value of the crop at any one time.

V. Summary

1. Quantitative surveys of marine infaunal benthos were made in north Baffin Island during 1954 and 1955, in Greenland during 1956, and in Foxe Basin during 1957.

2. Species found in each area are tabulated, and mean numbers and weights per sq. m. calculated. Lamellibranchs represented between 60 per cent and 80 per cent of the crop in north Baffin Island and near Godthaab, but were partially replaced by polychaetes in shallow water in Disko Bugt. Lamellibranchs were an insignificant part of the crop in the deepest Disko Bugt collections, where either foraminifera or polychaetes dominated. In Frustration Bay, Foxe Basin, echinoderms represented 72 per cent of the crop. The ratio between the amount of dry organic matter and fresh weight of animals varied with the taxonomic groups. The weight of dry organic matter in shallow water (to 50 metres depth) was generally 15 gm. per sq. m. or more, and in deeper water generally 10 gm. per sq. m. or less.

3. There are many possible sources of error in making quantitative surveys of the benthos, e.g., variable penetration by the sampler, and these are difficult to eradicate with present methods of collecting specimens. The collecting errors cause the recorded numbers and weights of animals per haul to vary; but some of the numerical variation also arises from natural phenomena, e.g., grouping of animals. The variation in the quantitative data is so great that they cannot be used as reliable estimates of the numbers and weights of animals per unit area of bottom. But the results of these surveys do show which species occur in a large percentage of the hauls and hence are widely spread in the investigated regions, which species are numerous in the hauls and hence are scattered densely over the bottom, and which species occur as heavy

ividuals or groups and hence contribute much to the total weight of animals unit area. The surveys reveal the marine level-bottom communities present l provide rough estimates of the standing crops of animals within the com- nities.

4. *Macoma* communities occurred in north Baffin Island on mud, sandy- d, and sand between 5–20 metres depth, in Godthaab Fjord on a sandy bank 10 metres depth, and in Disko Bugt on sand at 5–10 metres depth in Fortune- and Mudderbugt and in mud at 10 metres depth in Egedesminde harbour.

5. The *Macoma* communities in north Baffin Island appeared to change foraminifera communities between 20 and 50 metres depth (the maximum pled). In Laksebugt, Disko Bugt, a similar change occurred between 30 50 metres. The foraminifera community there extended to at least 170 tres.

6. An unusual faunal association occurred on mud bottom at 400 metres th on the prawn trawling grounds in Disko Bugt, and could not be referred any known community.

7. A *Chiridothea sabini* community was found in Foxe Basin, at Frustra- n Bay, and was best developed on mud bottom at 20 and 30 metres depth.

8. Dredge hauls near Igloolik in Foxe Basin suggested the presence of a *rtlandia arctica* community on mud at 22 metres depth.

9. *Macoma* communities appear to be very widely spread in shallow water about 50 metres depth in arctic North America, being replaced by others inly on localized, unusual substrates. An infaunal change with depth also pears to be widespread, with shallow-water communities being replaced by ers below 50 metres.

10. Periodic fluctuations in the composition of arctic marine level-bottom nmunities appear to be small.

11. The benthic fauna in regions with changing or fluctuating environ- ntal conditions tends to be a mixture of the species characterizing the pre- ling and the existing conditions.

12. The effects of substrate on arctic shallow-water infauna can usually described within the known limits of variability of the *Macoma* communities. casionally substrate affects the composition of the fauna so greatly in shallow ter, that the resulting species association justifies being regarded as a distinct nmunity, e.g., the *Portlandia arctica* and *Gomphina fluctuosa* communities mud and sand respectively.

13. Standing crop estimates for *Macoma* communities near the open sea erally indicate more than 1,000 animals (retained by 1- to 2-mm. sieves) per m. weighing more than 200 gm. per sq. m. Special conditions may reduce crops, e.g., at the heads of fiords, in very shallow water 0–5 metres depth, d on stagnant bottom. In north Baffin Island sandy-mud appeared to support ater crops than either mud or sand, possibly due to the proximity of this e of sediment to river mouths, which would develop a locally high organic ntent in the sediments.

14. The upper 200–300 metres of water throughout most of arctic North nerica originates in the Polar Basin. It has negative temperatures (high- tic conditions) and low salinities near 32‰. It overlies warmer, more saline

water of Atlantic origin. Summer insolation causes temperatures to rise i the uppermost 50 metres, occasionally to positive values (low-arctic conditions) This occurs especially at the surface where a warm brackish layer (the "fjord water") may develop. High-arctic temperatures may occur permanently at th surface in the farthest north regions. The terms "low-arctic" and "high-arctic can apply to temperature zones in the sea as well as zoogeographical regions.

15. In west Greenland and southeast Baffin Island polar water mixes wit Atlantic surface water (subarctic regions). Where the prevailing temperatur are positive, low-arctic conditions may be permanent, not seasonal.

16. The oceanographic conditions influence the infauna through thre dimensions, and its effects are considered under three categories—depth, latitud and combined depth and latitude effects.

17. Oceanographic effects with depth are as follows: The "fjord-water layer supports very few animals; it may be absent or extend to about 5 metre depth. *Macoma* and other shallow-water communities, often with large crop occur to about 50 metres depth, coinciding approximately with the extent o insolation effects in polar water. Deeper foraminifera and *Astarte crenat* communities, with small crops, occur below the shallow-water communities. I two regions where surface insolation is negligible, only *Chiridothea sabi* communities have been found in shallow water. The deep-water communitie have smaller crops (by weight) than the shallow-water communities, but swarm of benthic foraminifera in deep water may keep the density of animals as hig as in shallow water.

18. Oceanographic effects with latitude are as follows: Many infaun species are circumpolar but some are limited to only parts of arctic Nort America. Some are found only in the northern regions in polar water, others i southern regions with mixed polar and non-polar water (Atlantic or Pacific) The region between Victoria Island and north Baffin Island appears to be zoogeographic boundary between Atlantic and Pacific influences on th American arctic fauna. Differences in infaunal composition under polar an mixed polar and non-polar water nevertheless appear to be slight, and esse tially similar communities occur on equivalent substrates under the two sets o oceanographic conditions.

19. Combined depth and latitude effects are as follows: *Macoma* com munities, which in polar water appear bound to shallow water and its season low-arctic temperatures, may extend deeper in mixed polar and non-pola water, due to warmer water at greater depth. The arctic deep-water com munities have been found in polar water, and its underlying water of Atlanti origin; also in mixed polar and non-polar water. The deep communities ar apparently little affected by the oceanographic change from arctic to subarcti regions. The size of the infaunal crop also seems unaffected by the chang from Arctic to Subarctic because the known size of standing crops is as larg in the Arctic as in warmer regions.

20. The long life of the dominating lamellibranchs, the small number o species reproducing by pelagic larvae, and the low weight of predators a indicate a lower rate of infaunal production in the Arctic than in warmer marin regions.

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VII. Appendix: An Annotated List of Species Collected in the Survey

This list shows the epifauna and infauna collected and gives details of the origin and depth ranges not shown in Tables 2–6, which are based only on the larger hauls used for quantitative analyses. The taxonomic arrangement and nomenclature are based on recent usage in Greenland to facilitate comparison of Greenlandic and Canadian faunas.

Foraminifera: Several species. Arctic Bay 26–48 m., Eclipse Sound 35–55 m., Laksebugt 36–179 m., Igloolik 22 m.

Sponges: Several species. Epifauna. Arctic Bay 15–21 m., Laksebugt 88–152 m., Igloolik 22 m.

Coelenterates: Sea anemones and hydroid colonies. Epifauna. Arctic Bay 8–17 m., Eclipse Sound 8 m., Godthaab Fjord 10 m., Laksebugt 90–159 m., Igloolik 22 m.

Bryozoa: Several species. Epifauna. Laksebugt 88–159 m., Igloolik 22 m., Frustration Bay 30 m.

Nemertines: *Parapolia* sp., *Micrura* sp., *Cerebratulus* sp., and possibly others. Arctic Bay 2–26 m., Eclipse Sound 35–43 m., Disko Bugt 400–40 m., Igloolik 22 m.

Nemertodes: Igloolik 22 m.

Gephyrea: Several species. Arctic Bay 2–35 m., Eclipse Sound 2.5–47 m., Disko Bugt 440 m., Igloolik 22 m.

Priapulus sp.: Igloolik 22 m.

Errant polychaetes:

Gattyana cirrosa (Pallas): Arctic Bay 15–45 m., Eclipse Sound 25–40 m.

Harmothoe spp.: *Harmothoe imbricata* (L.) and other species. Arctic Bay 2–. m., Eclipse Sound 16–35 m.

(Other) Polynoid polychaetes: unidentified rare species or fragments. Godthaab Fjord 10 m., Fortunebay 5–10 m., Egedesminde harbour 5–10 m., Mudderbug 5–10 m., Laksebugt 36–159 m., Disko Bugt 380–410 m., Igloolik 22, 50 m., Frustration Bay 5–30 m.

Pholoe minuta (Fabricius): Arctic Bay 7–20 m., Eclipse Sound 5–47 m., Godthaab Fjord 10 m., Fortunebay 5–10 m., Egedesminde harbour 5–10 m., Mudderbug 5–10 m., Laksebugt 36–91 m., Frustration Bay 15 m.

Phyllodoce spp.: *Phyllodoce groenlandica* Oersted and other species. Arctic Bay 7–48 m., Eclipse Sound 5–40 m., Godthaab Fjord 10 m., Fortunebay 5–10 m., Mudderbugt 5 m., Laksebugt 36–167 m., Frustration Bay 5–20 m.

Eteone spp.: *Eteone longa* (Fabricius) and *Eteone flava* (Fabricius). Arctic Bay 2–48 m., Eclipse Sound 3–47 m., Godthaab Fjord 10 m., Fortunebay 5–10 m., Mudderbugt 5–10 m., Laksebugt 36–59 m., Igloolik 22 m., Frustration Bay 5 m.

(Other) Phyllodocid polychaetes: Unidentified rare species. Laksebugt 59 m., Frustration Bay 30 m.

Nereis zonata Malmgren: Arctic Bay 8–21 m.

Nephthys spp.: *Nephthys ciliata* Müller, *Nephthys coeca* Fabricius and other species. Arctic Bay 26–41 m., Eclipse Sound 26–55 m., Godthaab Fjord 10 m., Fortunebay 5–10 m., Egedesminde harbour 5–10 m., Mudderbugt 5–10 m., Laksebugt 41–166 m., Igloolik 50 m., Frustration Bay 3–30 m.

Onuphis conchylega Sars: Arctic Bay 48 m., Laksebugt 63–166 m.

Lumbrinereis spp.: *Lumbrinereis fragilis* (Müller) and other species. Arctic Bay 35–45 m., Laksebugt 82–179 m., Disko Bugt 380–440 m.

(Other) Errant polychaetes: Unidentified rare species or fragments. Arctic Bay 2–48 m., Eclipse Sound 5–47 m., Godthaab Fjord 10 m., Fortunebay 5–10 m.

gedesminde harbour 5–10 m., Mudderbugt 5–10 m., Laksebugt 35–166 m., rustration Bay 30 m.

tary polychaetes:

loplos armiger (Müller): Arctic Bay 7–41 m., Eclipse Sound 3–35 m., Godthaab jord 10 m., Mudderbugt 5–10 m., Laksebugt 45–52 m., Disko Bugt 380 m.

ther) Aricid polychaetes: *Nainereis quadricuspida* (Fabricius) and unidentified ragments. Fortunebay 5–10 m., Mudderbugt 5 m., Laksebugt 86–124 m., Frustration Bay 15 m.

libregma inflatum Rathke: Arctic Bay 2–41 m., Eclipse Sound 5 m.

wisia forbesii Johnston: Eclipse Sound 2–8 m., Godthaab Fjord 10 m., Frustration Bay 5 m.

helia sp.: Probably *Ophelia limacina* (Rathke). Eclipse Sound 2–8 m., Godthaab Fjord 10 m., Fortunebay 5–10 m.

rmotrypane aulogaster Rathke: Arctic Bay 7–41 m., Godthaab Fjord 10 m., gloolik 22, 50 m.

pitella capitata (Fabricius): Arctic Bay 2–20 m., Eclipse Sound 2 m.

tomastus latericeus Sars: Disko Bugt 380–440 m.

axillella gracilis (Sars): Disko Bugt 380–440 m.

ther) Maldanid polychaetes: Several species. Arctic Bay 7 m., Igloolik 22, 50 m., Frustration Bay 20–30 m.

wenia fusiformis Delle Chiaje: Arctic Bay 7–45 m., Eclipse Sound 6–55 m., Laksebugt 35–159 m.

yriochele heeri Malmgren: Arctic Bay 16–48 m., Eclipse Sound 3–55 m., Laksebugt 36–179 m.

stenides spp.: *Cistenides granulata* (L.) and *Cistenides hyperborea* Malmgren, which may not be distinct species (Wesenberg-Lund, 1950b). Many of the specimens cannot be identified as either species. Arctic Bay 7–45 m., Eclipse Sound 5–47 m., Godthaab Fjord 10 m., Fortunebay 5–10 m., Egedesminde harbour 5–10 m., Mudderbugt 5–10 m., Laksebugt 35–166 m., Disko Bugt 380 m., Igloolik 22, 50 m., Frustration Bay 20–30 m.

erebellides stroemi Sars: Arctic Bay 7 m., Eclipse Sound 25 m., Disko Bugt 380 m.

chone analis (Krøyer): Arctic Bay 2 m., Eclipse Sound 3–26 m., Godthaab Fjord 10 m., Fortunebay 5–10 m.

Other) Sabellid polychaetes: *Chone duneri* Malmgren and other species. Arctic Bay 41–5 m., Eclipse Sound 3 m., Igloolik 22 m., Frustration Bay 3 m.

Other) Sedentary polychaetes: Unidentified rare species and fragments. Arctic Bay 2–48 m., Eclipse Sound 2–55 m., Godthaab Fjord 10 m., Fortunebay 5–10 m., Egedesminde harbour 5–10 m., Mudderbugt 5–10 m., Laksebugt 36–179 m., Disko Bugt 380–440 m., Igloolik 22, 50 m., Frustration Bay 3–30 m.

ch: Unidentified single specimen. Igloolik 22 m.

her) Unidentified worms: fragments of nemertines, gephyrea, polychaetes, etc. odthaab Fjord 10 m., Fortunebay 5–10 m., Mudderbugt 5–10 m., Laksebugt 36–159 ., Disko Bugt 380–440 m.

stacea:

lyas araneus L.: Epifauna. Laksebugt 52–179 m.

lyas coarctatus Leach: Epifauna. Mudderbugt 5–10 m., Laksebugt 45–90 m.

lyas spp. (juv.): Epifauna. Unidentified specimens of the two preceding species. Godthaab Fjord 10 m.

nomuran decapods: Few unidentified specimens. Laksebugt 42–179 m., Igloolik 50 m.

Other) Decapod crustacea: Epifauna. *Spirontocaris* spp. and *Nectocrangon* sp. Godthaab Fjord 10 m., Mudderbugt 5–10 m., Laksebugt 35–103 m., Igloolik 22, 50 m., Frustration Bay 20–30 m.

Anonyx nugax (Phipps): Arctic Bay 16 m., Eclipse Sound 5–40 m., Godth
Fjord 10 m., Mudderbugt 5–10 m., Igloolik 22, 50 m., Frustration Bay 30 m.
(Other) Lysiannassid amphipods: *Onisimus* spp., *Pseudalibrotus* spp., and poss
others. Arctic Bay 16 m., Eclipse Sound 1–7 m.
Ampeliscid amphipods: *Ampelisca* spp., *Byblis* spp., *Haploops* spp., and poss
others. Arctic Bay 7–48 m., Eclipse Sound 16–47 m.
Pontoporeia femorata Krøyer: Eclipse Sound 35 m., Fortunebay 5–10 m., Mud
bugt 5 m.
Oedicerotid amphipods: *Paroediceros* spp., *Monoculodes* spp., and possibly oth
Arctic Bay 7–16 m., Eclipse Sound 5–49 m.
Gammarus spp.: Epifauna. *Gammarus setosus* Dementieva, *Gammarus zadd*
Sexton, subsp. *oceanicus* Segerstråle, and possibly others. Eclipse Sound 1
Mudderbugt 5–10 m.
Caprella septentrionalis Krøyer: Epifauna. Godthaab Fjord 10 m.
Neohela monstrosa (Boeck): Disko Bugt 440 m.
(Other) Amphipods: Unidentified rare species and fragments. Arctic Bay 7–26
Eclipse Sound 2–47 m., Godthaab Fjord 10 m., Fortunebay 5–10 m., Egedesmi
harbour 5–10 m., Mudderbugt 5–10 m., Laksebugt 37–175 m., Disko Bugt 380–
m., Igloolik 22, 50 m., Frustration Bay 5–30 m.
Chiridothea sabini (Krøyer): Igloolik 22, 50 m., Frustration Bay 15–30 m.
(Other) Isopods: Several species. Arctic Bay 20 m., Igloolik 22, 50 m.
Copepods: Plankton. Mudderbugt 5–10 m.
Cumacea: *Diastylis* spp., *Brachydiastylis* spp., *Campylaspis* spp., and possibly oth
Arctic Bay 20–38 m., Eclipse Sound 5–43 m., Fortunebay 5–10 m., Mudderbu
5–10 m., Disko Bugt 380 m., Igloolik 22, 50 m., Frustration Bay 3 m.
Philomedes globosus (Lilljeborg): Arctic Bay 10–48 m., Eclipse Sound 25–47
Fortunebay 5–10 m., Laksebugt 36 m.
Balanus balanus (L.): Epifauna. Arctic Bay 26 m.
Balanus spp.: Epifauna. *Balanus balanoides* (L.) and possibly *Balanus balanus* (L
and *Balanus crenatus* Bruguiere. Godthaab Fjord 10 m., Mudderbugt 5–10
Laksebugt 88–103 m., Frustration Bay 10–15 m.
Nebalia bipes Fabricius: Godthaab Fjord 10 m., Fortunebay 5–10 m., Egedesmin
harbour 5–10 m., Laksebugt 159 m., Igloolik 50 m.
Chaetonymphon sp.: Epifauna. Possibly *Chaetonymphon spinosissimum* Norm
Eclipse Sound 16 m.
(Other) Pycnogonids: Igloolik 22 m.

Brachiopods: Igloolik 50 m.

Lamellibranch molluscs:
Nucula tenuis (Montagu): Arctic Bay 7–41 m., Eclipse Sound 41 m., Laksebu
36–179 m., Igloolik 22, 50 m., Frustration Bay 30 m.
Nucula delphinodonta Mighels and Adams: Eclipse Sound 35–55 m.
Nucula ? sp.: Rare, unidentified specimens. Fortunebay 5–10 m.
Leda minuta Müller: Arctic Bay 8–48 m., Eclipse Sound 35 m., Laksebugt 82–175 m
Frustration Bay 15–30 m.
Leda pernula Müller: Lakesbugt 116–67 m., Igloolik 22, 50 m., Frustration Ba
20–30 m.
Portlandia arctica Gray: Igloolik 22 m.
Portlandia intermedia Sars: Disko Bugt 380–440 m., Igloolik 50 m.
Portlandia sp. juv.: unidentifiable juveniles. Disko Bugt 380–440 m.
Yoldia sp.: *Yoldia hyperborea* (Loven) Torell but possibly others also. Igloo
22, 50 m., Frustration Bay 20–30 m.
Arca glacialis Gray: Disko Bugt 380–440 m.
Dacrydeum vitreum (Møller): Eclipse Sound 31 m.
Crenella decussata Montagu: Laksebugt 63–166 m.

diolaria laevigata (Gray): Epifauna. Frustration Bay 15 m.
diolaria corrugata Stimpson: Arctic Bay 7–35 m., Laksebugt 46 m., Igloolik 0 m.
diolaria nigra (Gray): Arctic Bay 7–45 m., Eclipse Sound 19–35 m., Igloolik 2, 50 m.
diolaria faba (Müller): Epifauna. Fortunebay 5–10 m., Frustration Bay 15 m.
diolaria sp(p). (juv.): *M. corrugata*, *M. nigra*, and possibly others. Arctic Bay 0 m., Mudderbugt 5–10 m., Frustration Bay 20 m.
tilus edulis L.: Epifauna. Godthaab Fjord 10 m., Fortunebay 5–10 m.
ten groenlandicus Sowerby: Eclipse Sound 26 m.
ten islandicus Müller: Laksebugt 88–103 m.
tarte borealis (Chemnitz): Arctic Bay 7–45 m., Eclipse Sound 5–47 m., Igloolik 2, 50 m.
tarte montagui (Dillwyn): Arctic Bay 7–48 m., Eclipse Sound 11–55 m., Laksebugt 57–90 m., Igloolik 22, 50 m.
tarte elliptica Brown: Arctic Bay 7–32 m., Eclipse Sound 25–55 m., Laksebugt 90 m.
tarte spp. (juv.): Eclipse Sound 1–11 m., Laksebugt 152–66 m., Igloolik 22 m.
yasira gouldii Philippi: Arctic Bay 7–45 m., Eclipse Sound 5–55 m.
yasira sp(p).: At least two species, one of which may be *T. gouldii*. Laksebugt 36–179 m., Disko Bugt 380–440 m., Frustration Bay 15–30 m.
inopsis orbiculata Sars: Arctic Bay 7–16 m., Eclipse Sound 5–55 m., Godthaab Fjord 10 m., Fortunebay 5–10 m., Mudderbugt 5–10 m., Laksebugt 36–59 m., Frustration Bay 3–30 m.
rripes groenlandicum (Chemnitz): Arctic Bay 7–16 m., Eclipse Sound 5–25 m., Godthaab Fjord 10 m., Fortunebay 5–10 m., Egedesminde harbour 5–10 m., Mudderbugt 5–10 m., Laksebugt 36–46 m., Igloolik 50 m., Frustration Bay 15–30 m.
rdium ciliatum (Fabricius): Arctic Bay 16–26 m., Eclipse Sound 26–41 m., Laksebugt 45–179 m., Igloolik 50 m.
rdium elegantulum (Møller): Laksebugt 90 m.
acoma calcarea (Chemnitz): Arctic Bay 2–48 m., Eclipse Sound 8–47 m., Godthaab Fjord 10 m., Fortunebay 5–10 m., Egedesminde harbour 5–10 m., Laksebugt 90–124 m., Igloolik 22, 50 m.
acoma moesta (Deshayes): Arctic Bay 7–32 m., Eclipse Sound 8–47 m., Igloolik 22 m.
acoma spp. (juv.): Unidentifiable small specimens of the two preceding species. Arctic Bay 7–48 m., Eclipse Sound 5–41 m., Mudderbugt 5–10 m., Laksebugt 36–179 m., Igloolik 22 m., Frustration Bay 15–30 m.
axicava arctica L.: Epifauna. Arctic Bay 7–26 m., Eclipse Sound 5–41 m., Godthaab Fjord 10 m., Fortunebay 5–10 m., Mudderbugt 5–10 m., Laksebugt 28–90 m., Igloolik 22, 50 m.
ya truncata L.: Arctic Bay 7–38 m., Eclipse Sound 5–43 m., Godthaab Fjord 10 m., Fortunebay 5–10 m., Egedesminde harbour 5–10 m., Mudderbugt 5–10 m., Laksebugt 36–103 m., Igloolik 22 m.
yonsia arenosa Møller: Arctic Bay 16 m., Eclipse Sound 11 m.
hracia myopsis Beck: Eclipse Sound 10–35 m.
hracia septentrionalis Jeffreys: Eclipse Sound 5–8 m.
hracia spp.: Possibly the two preceding species. Godthaab Fjord 10 m., Mudderbugt 5–10 m., Laksebugt 90 m.
eriploma abyssorum Bush: Arctic Bay 26–32 m.
(Other) Lamellibranchs (juv.): A few rare species. Eclipse Sound 5 m., Godthaab Fjord 10 m.

cophoran molluscs:
onicella marmorea (Fabricius): Epifauna. Arctic Bay 21–32 m.

(Other) Placophora: Epifauna. Possibly *Trachydermon albus* (L.). Lakseb t 41–88 m., Igloolik 22 m.

Scaphopod molluscs:
Siphonodentalium vitreum Sars: Disko Bugt 440 m.

Solenogastrid molluscs:
Chaetoderma nitidulum Loven: Disko Bugt 380–440 m.
Chaetoderma sp.: Possibly aberrant *C. nitidulum.* Disko Bugt 380–440 m.
(Other) Solenogastres: Igloolik 22 m.

Gastropod molluscs:
Diaphana minuta Brown: Godthaab Fjord 10 m.
Cylichna alba (Brown): Arctic Bay 16–45 m., Eclipse Sound 10–40 m.
Cylichna occulta (Mighels): Arctic Bay 10–32 m., Eclipse Sound 5–43 m.
Cylichna spp.: Possibly the two preceding species. Igloolik 22 m., Frustration B 20–30 m.
Retusa obtusa (Montagu): Arctic Bay 26 m., Fortunebay 5–10 m., Laksebugt 45- m.
Philine lima (Brown): Arctic Bay 38 m.
(Other) Opisthobranchs: Igloolik 22 m., Frustration Bay 30 m.
Puncturella noachina (L.); Epifauna. Laksebugt 103 m., Igloolik 22 m.
Acmaea testudinalis (Müller): Epifauna. Arctic Bay 8–15 m., Godthaab Fjo 10 m.
Acmaea rubella (Fabricius): Epifauna. Arctic Bay 8–15 m., Igloolik 22 m.
Lepeta caeca (Müller): Epifauna. Arctic Bay 15–48 m., Laksebugt 90–159 m.
Margarita groenlandica (Chemnitz): Epifauna. Arctic Bay 21 m., Laksebugt 88 r Igloolik 22 m.
Margarita helicina (Phipps): Epifauna. Arctic Bay 16 m., Godthaab Fjord 10 r Fortunebay 5–10 m., Igloolik 22 m.
Margarita cinerea (*Couthouy*): Epifauna. Laksebugt 82 m.
Margarita spp.: Epifauna. Igloolik 22, 50 m.
Littorina saxatilis L.: Epifauna. Godthaab Fjord 10 m.
Cingula castanea (Møller): Arctic Bay 7–26 m., Mudderbugt 5–10 m.
Cingula spp.: Several species, including possibly *Cingula globulus* (Møller Godthaab Fjord 10 m., Mudderbugt 5–10 m., Laksebugt 45–59 m.
Onoba aculeus (Gould): Fortunebay 5–10 m.
Turritella erosa Couthouy: Laksebugt 36–124 m.
Trichotropis borealis Broderip and Sowerby: Arctic Bay 10–26 m., Eclipse Sour 10 m., Igloolik 22 m., Frustration Bay 15–20 m.
Lunatia pallida (Broderip and Sowerby): Arctic Bay 20–35 m., Eclipse Soun 11–35 m., Laksebugt 90–124 m., Igloolik 50 m.
Natica clausa Broderip and Sowerby: Laksebugt 45 m.
Velutina velutina (Müller): Laksebugt 88 m.
Trophon clathratus (L.): Laksebugt 88–124 m.
Buccinum belcheri Reeve: Eclipse Sound 35 m.
Buccinum ciliatum Fabricius: Eclipse Sound 5 m.
Buccinum spp.: Godthaab Fjord 10 m., Laksebugt 42–166 m., Igloolik 22, 50 m Frustration Bay 15 m.
Bela nobilis (Møller): Arctic Bay 7 m., Mudderbugt 5–10 m., Laksebugt 35–124 m
Bela exarata (Møller): Eclipse Sound 35 m.
Bela violacea (Mighels): Arctic Bay 10–20 m., Eclipse Sound 3–35 m., Mudderbug 5–10 m., Laksebugt 46 m.
Bela simplex Middendorff: Laksebugt 90 m.
Bela decussata (Couthouy): Arctic Bay 7 m., Eclipse Sound 5–26 m.
Bela spp.: Godthaab Fjord 10 m., Laksebugt 52–175 m., Disko Bugt 400–40 m Igloolik 22, 50 m., Frustration Bay 3–30 m.

(her) Gastropods: A few rare species. Godthaab Fjord 10 m., Igloolik 22, 50 m., rustration Bay 5–20 m.

oderms:

)iura sarsi Lütken: Arctic Bay 41 m., Eclipse Sound 6–35 m., Laksebugt 41–98 m., gloolik 22, 50 m.

)iura robusta (Ayres): Arctic Bay 15–38 m., Eclipse Sound 5–55 m., Mudderugt 5–10 m., Laksebugt 41–103 m.

)iocten sericeum (Forbes): Arctic Bay 20–48 m., Eclipse Sound 11–43 m., gloolik 50 m.

(ther) Ophiuroids: Fortunebay 5–10 m., Mudderbugt 5–10 m., Laksebugt 88–56 m., Igloolik 22, 50 m., Frustration Bay 3–30 m.

mgylocentrotus droebachiensis (Müller): Epifauna. Arctic Bay 7–26 m., aksebugt 57–103 m., Igloolik 22 m.

umaria calcigera (Stimpson): Arctic Bay 16–32 m., Eclipse Sound 25–33 m., Godthaab Fjord 10 m., Mudderbugt 5–10 m., Laksebugt 45–61 m., Frustration ay 15–30 m.

lus phantapus (Strussenfeldt): Arctic Bay 45 m.

lus fabricii (Duben and Koren): Arctic Bay 21 m., Laksebugt 57–103.

lus sp.: Igloolik 22, 50 m.

pyrgus scaber Lütken: Eclipse Sound 33–43 m., Frustration Bay 20–30 m.

riotrochus rinki Steenstrup: Arctic Bay 7–26 m., Eclipse Sound 5–35 m., Frustration Bay 20 m.

(ther) Holothurians: Godthaab Fjord 10 m., Egedesminde harbour 5–10 m., aksebugt 46–90 m.

nodiscus crispatus (Retzius): Disko Bugt 380 m.

(ther) Asteroids: Fortunebay 5–10 m., Laksebugt 28 m., Igloolik 22 m., Frustration Bay 30 m.

cates:

onaia corrugata Goodsir and Forbes: Eclipse Sound 5–35 m., Mudderbugt 5–10 n., Laksebugt 45–52 m., Frustration Bay 30 m.

)ther) Tunicates: Epifauna. Arctic Bay 2 m., Laksebugt 82–152 m., Igloolik 22, 50 m., Frustration Bay 15 m.

s

lus bicornis (Reinhardt): Epifauna. Eclipse Sound 10 m.

ymnacanthus sp.: Epifauna. Possibly *Gymnacanthus tricuspis* (Reinhardt). Arctic Bay 7 m.

)ther) Sculpins: Epifauna. Igloolik 22, 50 m.

)ther) Fish: Epifauna. Eclipse Sound 8 m., Fortunebay 5–10 m., Frustration Bay 20 m.

Table 1. Collecting grounds in Disko Bugt.

Series no.	Locality	No. of hauls	Depth in metres	Substrate
1	Fortunebay	13	5–10	Sand and alga (kelp and rockweed)
2	Egedesminde harbour	10	5–10	Soft mud with rotting vegetation and domes sewage
3a	Mudderbugt	12	5–10	Hard sand with broken shells overlain quarter-inch layer of river mud
b	Mudderbugt	3	3	Soft mud close to river mouth
c	Mudderbugt	6	3–5	Series between 3a and 3b
4	Laksebugt	14	28–63	Sticky mud with sand, pebbles, and she plus 1 haul from soft mud in Godhavn harbo
5	Laksebugt	14	82–124	Mud, many small stones, and broken shells
6	Laksebugt	7	151–79	Mud, many small stones, and broken shells
7	69° 15N., 53° 10W.	5	380–440	Soft mud

Table 2. Quantitative data for infauna collected in north Baffin Island.

metres	0–3			5–14			5–47			7–55		
e	Sand			Sand			Sandy-mud			Mud		
auls	11			8			23			20		
pecies	Present in % of hauls	Mean no./sq. m.	Mean wt./sq. m. in gm.	Present in % of hauls	Mean no./sq. m.	Mean wt./sq. m. in gm.	Present in % of hauls	Mean no./sq. m.	Mean wt./sq. m. in gm.	Present in % of hauls	Mean no./sq. m.	Mean wt./sq. m. in gm.
ifera	—	—	—	—	—	—	30	85	—	50*	151*	3
nes	9	1	—	—	—	—	13	1	—	5	1	—
a	18	3	—	11	8	—	17	3	—	15	3	—
a cirrosa	—	—	—	—	—	—	4	1	—	15	3	—
oe spp.	18	4	5*	—	—	—	4	—	—	5	1	—
minuta	—	—	—	44	8	—	61*	27	—	30	7	—
ce spp.	—	—	—	11	1	—	30	10	2	30	6	—
spp.	18	3	—	56*	13	—	35	11	—	25	4	—
onata	—	—	—	—	—	—	—	—	—	15	3	—
s spp.	—	—	—	—	—	—	26	4	5	20	3	—
conchylega	—	—	—	—	—	—	—	—	—	5	3	—
ereis spp.	—	—	—	—	—	—	—	—	—	15	3	2
rant polychaetes	18	7	—	11	1	—	61*	15	2	45	11	—
s armiger	9	1	—	22	7	—	43	11	—	40	10	—
gma inflatum	18	8	—	11	1	—	13	10	—	5	1	—
forbesii	46	11	5*	22	11	3	9	3	2	—	—	—
sp.	45	11	3	44	22	2	13	3	—	—	—	—
ypane aulogaster	—	—	—	—	—	—	4	—	—	20	3	—
a capitata	18	6	—	—	—	—	—	—	—	5	1	—
id polychaetes	—	—	—	—	—	—	—	—	—	10	1	—
fusiformis	—	—	—	44	392*	18	61*	116*	10	40	125*	10
hele heeri	9	1	—	—	—	—	61*	60	1	60*	161*	2
ies spp.	—	—	—	—	—	—	52*	22	8	25	4	—
ides stroemi	—	—	—	—	—	—	4	—	—	5	3	—
e analis	18	17	8*	78*	204*	24*	17	251*	17	20	10	—
abellid polychaetes	27	255*	10*	—	—	—	—	—	—	10	3	—
edentary polychaetes	45	46	—	44	202*	2	91*	104*	1	75*	88	2
nugax	—	—	—	22	7	2	22	171*	42*	20	14	3
ysiannassid												
ipods	27	4	—	56*	8	—	22	66	1	—	—	—
scid amphipods	—	—	—	—	—	—	39	13	—	30	8	—
mesa femorata	—	—	—	—	—	—	4	—	—	—	—	—
otid amphipods	—	—	—	33	8	—	26	14	—	5	1	—
mphipods	9	1	—	11	13	—	13	3	—	15	3	—
a	—	—	—	—	—	—	4	—	—	—	—	—
ea	—	—	—	11	1	—	39	10	—	25	8	—
edes globosus	—	—	—	—	—	—	70*	115*	1	55*	83	1
tenuis	—	—	—	—	—	—	34	11	2	20	4	2
delphinodonta	—	—	—	—	—	—	9	1	—	10	3	—
inula	—	—	—	—	—	—	39	21	2	35	11	2
eum vitreum	—	—	—	—	—	—	—	—	—	5	1	—
aria corrugata	—	—	—	—	—	—	22	11	5	10	3	2
aria nigra	—	—	—	—	—	—	17	4	3	20	4	7
aria spp. juv.	—	—	—	—	—	—	4	1	—	—	—	—
groenlandicus	—	—	—	—	—	—	—	—	—	5	1	—
borealis	—	—	—	22	6	18	83*	56	123*	55*	17	20*
montagui	—	—	—	—	—	—	70*	221*	37	50*	46	15
elliptica	—	—	—	—	—	—	26	4	2	15	4	—
spp.	—	—	—	—	—	—	9	1	—	—	—	—
ra gouldii	—	—	—	—	—	—	61*	43	2	45	21	—
sis orbiculata	—	—	—	56*	20	—	61*	36	—	35	22	—
s groenlandicum	—	—	—	56*	13	74*	35	15	12	25	4	25*
m ciliatum	—	—	—	—	—	—	26	4	32	10	3	30*
ta calcarea	9	1	—	22	13	5	78*	38	24	75*	21	24*
na moesta	—	—	—	11	1	-	57*	13	3	20	6	—
ta spp. juv.	—	—	—	—	—	—	78*	62	2	30	11	—
uncata	—	—	—	67*	22	12	74*	41	40*	60*	14	25*
a arenosa	—	—	—	—	—	—	9	1	—	-	—	—
a myopsis	—	—	—	11	1	—	13	3	—	5	1	—
a septentrionalis	—	—	—	11	4	—	9	3	2	—	-	—
oma abyssorum	—	—	—	—	—	—	4	-	-	5	1	-
lamellibranchs	—	—	—	—	—	—	4	-	—	-	-	-
na alba	—	—	—	—	—	—	22	3	—	10	3	-
na oculta	—	—	—	56*	29	2	26	10	1	5	1	—
obtusa	—	—	—	—	—	—	4	-	—	-	-	-
clima	—	—	—	-	—	—	-	—	—	5	1	—
a castanea	—	—	—	—	—	—	22	11	—	—	—	—
inopis borealis	—	—	—	—	—	—	22	4	2	5	1	—
a pallida	—	—	—	—	—	—	22	3	—	5	1	—
um belcheri	—	—	—	—	—	—	4	1	5	—	—	—
um ciliatum	—	—	—	—	—	—	4	1	3	—	-	-
nobilis	—	—	—	—	—	—	-	—	—	5	1	—
exornata	—	—	—	-	—	—	4	—	—	—	—	—
tudacea	9	1	—	44	11	—	22	3	-	5	3	—
fe carinata	—	—	—	—	—	—	13	3	—	10	3	—
ra sarsi	—	—	—	33	6	27*	4	1	-	25	4	10
ra robusta	—	—	—	—	—	—	17	3	—	20	3	-
cten sericeum	—	—	—	—	—	—	22	8	2	60*	25	1
maria calcigera	—	—	—	—	—	—	26	6	32	—	—	—
phantapus	—	—	—	-	—	—	—	—	—	5	1	5
fabricii	—	—	—	—	—	—	—	—	—	5	1	—
gus wahlbergi	—	—	—	—	—	—	22	6	—	5	1	-
bus rinki	—	—	—	78*	49	12	57*	32	7	20	11	1
ata corrugata	—	—	—	—	—	-	13	1	3	15	3	8
Totals		381	31		1,082	201		1,815	438		987	200

dominant species (see p. 12).

Table 3. Quantitative data for infauna collected from shallow water in Godthaab Fjord and Disko Bugt.

Place	Godthaab Fjord			Fortunebay			Egedesminde harbour			Mudderbugt		
Depth in metres	10			5–10			5–10			5–10		
Substrate	Sand			Sand			Mud			Sand		
No. of hauls	39			13			10			12		
Species	Present in % of hauls	Mean no./sq. m.	Mean wt./sq. m. in gm.	Present in % of hauls	Mean no./sq. m.	Mean wt./sq. m. in gm.	Present in % of hauls	Mean no./sq. m.	Mean wt./sq. m. in gm.	Present in % of hauls	Mean no./sq. m.	Me wt./ m in g
Polynoid polychaetes	92*	43	1	46	5	2	30	4	—	33	3	
Pholoe minuta	97*	162*	2	92*	75	1	70*	22	—	83*	101*	
Phyllodoce spp.	33	7	10	53*	8	9	—	—	—	—	—	
Eteone spp.	33	5	—	92*	232*	2	—	—	—	25	3	
Nephthys spp.	95*	30	9	46	8	20	60*	13	11	58*	8	
Other errant polychaetes	82*	169*	2	84*	357*	1	50*	11	—	8	1	
Scoloplos armiger	41	7	—	—	—	—	—	—	—	8	2	
Other Aricid polychaetes	—	—	—	31	25	—	—	—	—	—	—	
Travisia forbesii	13	2	1	—	—	—	—	—	—	—	—	
Ophelia sp.	64*	22	1	8	1	—	—	—	—	—	—	
Ammotrypane aulogaster	3	—	—	—	—	—	—	—	—	—	—	
Cistenides spp.	97*	190*	20*	100*	428*	151*	90*	251*	130*	100*	338*	1
Euchone analis	31	9	—	23	18	1	—	—	—	—	—	
Othersedentarypolychaetes	79*	79	2	54*	75	1	20	2	—	83*	110*	
Other unidentified worms	38	16	—	31	7	—	—	—	—	8	1	
Anonyx nugax	54*	20	1	—	—	—	—	—	—	33	5	
Pontoporeia femorata	—	—	—	8	1	—	—	—	—	—	—	
Other amphipods	95*	200*	2	38	8	—	30	83*	1	100*	262*	
Cumacea	—	—	—	8	1	—	—	—	—	17	3	
Philomedes globosus	—	—	—	15	8	—	—	—	—	—	—	
Nebalia bipes	92*	104*	1	8	1	—	30	7	—	—	—	
Nucula? sp.	—	—	—	23	30	1	—	—	—	—	—	
Modiolaria sp. juv.	—	—	—	—	—	—	—	—	—	92*	82	
Axinopsis orbiculata	85*	54	1	85*	130	1	—	—	—	100*	98*	
Serripes groenlandicum	13	1	12*	30	4	55*	40	4	14	83*	24	
Macoma calcarea	97*	347*	58*	77*	71	25*	50*	11	9	—	—	
Macoma sp. juv.	—	—	—	—	—	—	—	—	—	92*	203*	
Mya truncata	90*	93	6	85*	28	26*	70*	23	150*	100*	226*	
Thracia spp.	33	9	2	—	—	—	—	—	—	8	1	
Diaphana minuta	13	2	—	—	—	—	—	—	—	—	—	
Retusa obtusa	—	—	—	31	3	—	—	—	—	—	—	
Cingula castanea	—	—	—	—	—	—	—	—	—	17	12	
Cingula sp.	77*	145*	1	—	—	—	—	—	—	42	4	
Onoba aculeus	—	—	—	8	1	—	—	—	—	—	—	
Buccinum spp.	3	—	—	—	—	—	—	—	—	—	—	
Bela nobilis	—	—	—	—	—	—	—	—	—	33	3	
Bela violacea	—	—	—	—	—	—	—	—	—	50*	7	1
Bela spp.	62*	12	1	—	—	—	—	—	—	—	—	
Ophiura robusta	—	—	—	—	—	—	—	—	—	8	1	
Other ophiuroids	—	—	—	8	1	—	—	—	—	8	1	
Cucumaria calcigera	5	1	—	—	—	—	—	—	—	42	5	1
Other holothurians	—	—	—	—	—	—	10	1	5	—	—	
Other asteroids	—	—	—	8	2	—	—	—	—	—	—	
Pelonaia corrugata	—	—	—	—	—	—	—	—	—	25	3	2
Totals		1,729	133		1,528	296		432	320		1,507	48

*Dominant species (see p. 12).

Table 4. Quantitative data for infauna collected from deep water in Disko Bugt.

	Laksebugt	Laksebugt	Laksebugt	69°15N., 53°10W.
in metres	28–63	82–124	151–79	380–440
rate	Mud and sand	Mud and stones	Mud and stones	Soft mud
f hauls	14	14	7	5
Species	Present in % of hauls / Mean no./sq. m. / Mean wt./sq. m. in gm.	Present in % of hauls / Mean no./sq. m. / Mean wt./sq. m. in gm.	Present in % of hauls / Mean no./sq. m. / Mean wt./sq. m. in gm.	Present in % of hauls / Mean no./sq. m. / Mean wt./sq. m. in gm.
minifera	84• 396• 5•	100• 1386• 37•	86• 1092• 26•	— — —
rtines	— — —	— — —	— — —	40 10 1
yrea	— — —	— — —	— — —	20 2 —
oid polychaetes	38 5	31 9 1	14 1	60• 12 1
e minuta	62• 20	8 2 —	— — —	— — —
odoce spp.	8 1 —	— — —	14 1 —	— — —
e spp.	15 4 —	— — —	— — —	— — —
Phyllodocid chaetes	8 2 1	— — —	— — —	— — —
thys spp.	31 2 —	46 8 5	29 3 —	— — —
his conchylega	8 1 —	15 3 —	29 26 1	— — —
rinereis spp.	— — —	31 4 3	57• 7 1	40 12 —
r errant polychaetes	54• 8 1	23 4 —	14 1 —	— — —
plos armiger	15 4 —	— — —	— — —	20 2 —
r Aricid polychaetes	— — —	38 5 —	— — —	— — —
mastus latericeus	— — —	— — —	— — —	100• 68 29•
rillella gracilis	— — —	— — —	— — —	100• 18 5
ia tuusiformis	62• 85• 4	15 3 —	14 3 —	— — —
ochele heeri	46 18 —	31 5 —	86• 21 —	— — —
nides hyperborea	— — —	— — —	— — —	20 2 —
nides spp.	100• 48 11•	62• 13 2	14 1 —	— — —
ellides stroemi	— — —	— — —	— — —	20 2 1
r sedentary polychaetes	54• 22 1	85• 21 1	86• 43 5•	100• 500• 17•
r unidentified worms	31 6 3	8 1 —	14 1 —	40 72 1
nuran decapods	8 1 —	— — —	14 1 —	— — —
dia monstrosa	— — —	— — —	— — —	20 3 1
r amphipods	54• 12 —	38 5 —	57• 11 1	40 4 —
acea	31 6 —	— — —	— — —	20 4 —
medes globosus	8 8 —	— — —	— — —	— — —
dia bipes	— — —	— — —	14 1 —	— — —
da tenuis	23 4 —	54• 8 —	71• 9 —	— — —
minuta	— — —	31 4 —	57• 7 —	— — —
pernula	— — —	23 4 2	57• 11 2	— — —
ndia intermedia	— — —	— — —	— — —	40 16 1
anisa sp. juv.	— — —	— — —	— — —	40 10 —
glacialis	— — —	— — —	— — —	60• 58 4
da decussata	8 1 —	8 2 —	29 6 —	— — —
olaria corrugata	8 1 —	— — —	— — —	— — —
n islandicus	— — —	15 2 6	— — —	— — —
te montagui	8 1 —	8 1 —	— — —	— — —
te ciliplica	— — —	15 2 5	— — —	— — —
te spp.	— — —	— — —	29 3 —	— — —
stra spp.	8 8 —	8 2 —	57• 14 —	100• 104• 1
opsis orbiculata	8 5 —	— — —	— — —	— — —
pes groenlandicum	23 2 3	— — —	— — —	— — —
tium citisatum	15 2 1	54• 6 35•	29 4 9•	— — —
tium elegantulum	— — —	8 1 —	— — —	— — —
oma calcarea	— — —	23 2 1	— — —	— — —
oma sp. juv.	69• 18 1	38 5 —	29 10 —	— — —
truncata	8 2 7•	23 2 2	— — —	— — —
ta spp.	— — —	8 1 2	— — —	— — —
modentalium vitreum	— — —	— — —	— — —	20 2 —
derma nitidulum	— — —	— — —	— — —	80• 14 —
derma sp.	— — —	— — —	— — —	40 8 —
sa obtusa	15 2 —	8 1 —	— — —	— — —
ula spp.	23 3 —	— — —	— — —	— — —
tella crosa	23 3 1	54• 23 2	— — —	— — —
ta pallida	— — —	8 1 —	— — —	— — —
ea clausa	8 1 —	— — —	— — —	— — —
ina tellutina	— — —	8 1 —	— — —	— — —
ghon clathratus	— — —	15 3 1	— — —	— — —
anum spp.	23 3 2	8 1 4	14 1 —	— — —
nobilis	54• 24 1	62• 8 —	— — —	— — —
rudacea	8 1 —	— — —	— — —	— — —
simplex	— — —	8 1 —	— — —	— — —
spp.	8 1 —	— — —	14 1 —	60• 6 —
ura sarsi	8 1 2	8 2 —	— — —	— — —
ura robusta	8 1 —	8 5 2	— — —	— — —
ostyhiurotds	— — —	15 3 —	57• 14 1	— — —
amaria calcigera	23 9 1	— — —	— — —	— — —
lus fabricii	8 1 —	31 4 1	— — —	— — —
er holothurians	8 1 1	8 1 —	— — —	— — —
ostinus crispatus	— — —	— — —	— — —	20 2 7
nata corrugata	38 5 1	— — —	— — —	— — —
Totals	749 47	1,565 112	1,293 46	931 69

ominant species (see p. 12).

Table 5. Quantitative data for infauna collected in Frustration Bay.

Depth in metres	5			15			20			30		
Substrate	Sand			Sandy-mud			Mud			Mud		
No. of hauls	6			4			5			11		
Species	Present in % of hauls	Mean no./sq. m.	Mean wt./sq. m. in gm.	Present in % of hauls	Mean no./sq. m.	Mean wt./sq. m. in gm.	Present in % of hauls	Mean no./sq. m.	Mean wt./sq. m. in gm.	Present in % of hauls	Mean no./sq. m.	Mean wt./sq. m. in gm
Polynoid polychaetes	17	2	—	—	—	—	20	2	—	9	1	—
Pholoe minuta	—	—	—	50*	5	—	—	—	—	—	—	—
Phyllodoce spp.	50*	5	—	50*	8	1	20	2	—	—	—	—
Eteone spp.	17	3	—	—	—	—	—	—	—	—	—	—
Other Phyllodocid polychaetes	—	—	—	—	—	—	—	—	—	9	1	—
Nephthys spp.	67*	16	17*	100*	20*	9	100*	20	7	100*	46*	27*
Other errant polychaetes	—	—	—	—	—	—	—	—	—	9	2	—
Aricid polychaetes	—	—	—	25	5	—	—	—	—	—	—	—
Travisia forbesii	33	7	7*	—	—	—	—	—	—	—	—	—
Maldanid polychaetes	—	—	—	—	—	—	40	10	15*	45	25	5
Cistenides spp.	—	—	—	—	—	—	20	2	—	36	4	4
Sabellid polychaetes	83*	85*	9*	—	—	—	—	—	—	—	—	—
Other sedentary polychaetes	100*	80*	1	100*	28*	5	100*	92*	1	91*	106*	5
Anonyx nugax	—	—	—	—	—	—	—	—	—	9	1	—
Other amphipods	83*	15	—	50*	30*	2	—	—	—	9	1	—
Chiridotlea sabini	—	—	—	25	8	5	100*	12	9*	64*	9	6
Cumacea	100*	67*	1	—	—	—	—	—	—	—	—	—
Nucula tenuis	—	—	—	—	—	—	—	—	—	9	1	1
Leda minuta	—	—	—	25	3	1	—	—	—	18	1	—
Leda pernula	—	—	—	—	—	—	20	2	1	9	1	1
Yoldia sp.	—	—	—	—	—	—	20	2	2	73*	11	17
Modiolaria sp.	—	—	—	—	—	—	40	8	7	—	—	—
Thyasira sp.	—	—	—	25	3	1	—	—	—	18	3	—
Axinopsis orbiculata	—	—	—	—	—	—	20	2	—	9	1	—
Serripes groenlandicum	—	—	—	50*	5	132*	—	—	—	9	1	17
Macoma spp.	—	—	—	100*	48*	14	100*	12	3	82*	11	3
Cylichna sp.	—	—	—	—	—	—	40	4	—	36	4	—
Other opisthobranchs	—	—	—	—	—	—	—	—	—	9	1	—
Trichotropis borealis	—	—	—	25	3	2	20	2	1	—	—	—
Buccinum spp.	—	—	—	50*	5	23*	—	—	—	—	—	—
Bela spp.	—	—	—	50*	10	1	40	4	—	9	1	—
Other gastropods	17	2	—	—	—	—	20	2	—	—	—	—
Ophiuroids	—	—	—	100*	25*	5	80*	16	3	36	5	1
Cucumaria calcigera	—	—	—	25	3	9	60*	8	32*	82*	24	173*
Eupyrgus scaber	—	—	—	—	—	—	60*	6	1	18	1	—
Myriotrochus rinki	—	—	—	—	—	—	60*	8	11*	—	—	—
Asteroids	—	—	—	—	—	—	—	—	—	9	1	29*
Pelonaia corrugata	—	—	—	—	—	—	—	—	—	9	1	1
Totals		282	35		209	210		216	93		264	290

*Dominant species (see p. 12).

Table 6. Percentage of dredge hauls in which infaunal species were collected near Igloolik.

F.R.B. Stn. no.	723	719
Depth in metres	22	50
Substrate	Mud	Mud
No. of hauls	13	4
Species		
Foraminifera	8	—
Nemertines	46	—
Nemertodes	8	—
Priapulus sp.	8	—
Other gephyrea	8	—
Polynoid polychaetes	85*	50*
Eteone spp.	8	—
Nephthys spp.	—	25
Ammotrypane aulogaster	8	25
Maldanid polychaetes	8	25
Cistenides spp.	15	75*
Sabellid polychaetes	77*	—
Other sedentary polychaetes	92*	75*
Leech	8	—
Anomuran decapods	—	25
Anonyx nugax	62*	50*
Other amphipods	77*	50*
Chiridothea sabini	69*	50*
Other isopods	31	50*
Cumacea	15	25
Nebalia bipes	46	—
Pycnogonids	8	—
Brachiopods	—	50*
Nucula tenuis	62*	100*
Leda pernula	8	25
Portlandia arctica	100*	—
Portlandia intermedia	—	50*
Yoldia sp.	8	75*
Modiolaria corrugata	—	25
Modiolaria nigra	8	75*
Astarte borealis	46	75*
Astarte montagui	31	25
Astarte spp.	31	—
Serripes groenlandicum	—	75*
Cardium ciliatum	—	50*
Macoma calcarea	23	75*
Macoma moesta	8	—
Macoma spp.	8	—
Mya truncata	8	—
Solenogastres	8	—
Cylichna spp.	8	—
Other opisthobranchs	62*	—
Trichotropis borealis	15	—
Lunatia pallida	—	25
Buccinum spp.	15	50*
Bela spp.	8	25
Other gastropods	8	25
Ophiura sarsi	8	75*
Ophiocten sericeum	—	50*
Other ophiuroids	8	25
Psolus sp.	8	25
Asteroids	62*	—
Unidentified tissues	38	—

*Dominant species (see p. 12).

Table 7. Percentage composition of the fresh weight of various species.

Species	No. of specimens	Percentage of various components: water	residue after dehydration	dry organic matter	ash
A. Expressed as percentages of the total body weight (including any shells, tubes, etc.)					
Foraminifera	350	33	67	1	66
Nemertines	22	77	23	—	—
Polynoid polychaetes	30	80	20	—	—
Pholoe minuta	100	77	23	—	—
Phyllodoce spp.	1	79	21	20	1
"	8	75	25	23	2
Nephthys spp.	2	75	25	—	—
"	32	72	28	24	4
Cistenides spp.	36	91	9	—	—
" *	70	84	16	8	8
Sedentary polychaetes (tubeless)	50	78	22	—	—
" " (tube-dwellers)	100	50	50	4	46
Amphipods	300	64	36	17	19
"	50	78	22	14	8
Axinopsis orbiculata	100	43	57	—	—
*Serripes groenlandicum**	1	39	61	11	50
*Cardium ciliatum**	2	26	74	3	71
Macoma calcarea	300	52	48	10	38
" " *	12	33	67	9	58
" " *	13	33	67	—	—
Mya truncata	100	54	46	—	—
" " *	1	52	48	14	34
" " *	1	51	49	12	37
" " *	1	60	40	12	28
Mixed lamellibranchs	11	56	44	—	—
Gastropods†	50	42	58	10	48
Asteroids	1	76	24	—	—
"	1	76	24	—	—
B. Expressed as percentages of tissue weight (i.e., body weight without any shells, tubes, etc.)					
Cistenides spp.	70	70	30	14	16
Serripes groenlandicum	1	73	27	25	2
Cardium ciliatum	2	83	17	15	2
Macoma calcarea	12	75	25	21	4
Mya truncata	1	81	19	17	2
" "	1	76	24	21	3
" "	1	83	17	15	2

*Shells or tubes removed before dehydration.
†Mixed specimens of *Bela* spp., *Buccinum* spp., *Cingula* spp., and *Diaphana minuta*.

Table 8. Estimates of the amount of dry organic matter expressed as gm. per sq. m. in the investigated regions.

Species	North Baffin Island: Sand	Sandy-mud	Mud	Godthaab Fjord	Disko Bugt: Egedesminde Fortune-bay	Egedesminde harbour	Mudder-bugt	Laksebugt 50m.	Laksebugt 100m.	Laksebugt 170m.	Prawn grounds	Frustration Ba
Foraminifera	—	—	—	—	—	—	—	—	—	—	—	—
Errant polychaetes and other species without shells or tubes	1	3	2	5	7	2	2	1	2	1	11	1
Cistenides spp.	—	1	—	2	15	13	1	1	—	—	—	—
Tubicolous sedentary polychaetes	2	1	1	—	—	—	—	—	—	—	—	—
Crustacea	—	6	1	1	—	—	—	—	—	—	—	1
Lamellibranchs	11	30	15	8	11	17	2	1	5	1	1	4
Gastropods	—	1	—	—	—	—	—	1	1	—	—	—
Echinoderms	2	2	1	—	—	—	—	—	—	—	1	10
Total dry organic matter per sq. m.	16	44	20	16	33	32	5	4	8	2	13	16
Total fresh weight of animals per sq. m.	200	446	200	133	296	320	48	47	112	45	69	290
Ratio: Dry organic matter: fresh weight	1:13	1:10	1:10	1:8	1:9	1:10	1:10	1:12	1:14	1:23	1:5	1:18

Dredged from Gosforth Plain (seaward) in seven series each taken during one day between 9 May and 5 June 1956

Series no.	1			2			3			4			5			6			7		
No. of hauls	8			16			10			7			18			20			21		
Species	Present in % of hauls	Mean no./sq. m.	Mean wt./sq. m. in gm.	Present in % of hauls	Mean no./sq. m.	Mean wt./sq. m. in gm.	Present in % of hauls	Mean no./sq. m.	Mean wt./sq. m. in gm.	Present in % of hauls	Mean no./sq. m.	Mean wt./sq. m. in gm.	Present in % of hauls	Mean no./sq. m.	Mean wt./sq. m. in gm.	Present in % of hauls	Mean no./sq. m.	Mean wt./sq. m. in gm.	Present in % of hauls	Mean no./sq. m.	Mean wt./sq. m. in gm.
Pelagic polychaetes	63	46	2	75	31	1	70	24	1	86	41	1	83	61	2	95	52	2	95	38	1
Nereis virens	75	26	—	94	102	1	80	144	1	100	127	1	100	122	1	95	102	1	100	193	2
***Phyllodoce* spp.**	13	3	1	25	4	5	20	2	4	42	1	—	6	2	—	10	2	2	43	12	14
***Eteone* spp.**	38	4	—	19	3	—	70	11	—	42	4	—	11	1	—	10	1	—	48	5	—
***Nephthys* spp.**	88	25	8	75	16	4	90	23	7	86	30	7	94	35	12	95	34	9	90	24	8
Other errant polychaetes	75	169	1	69	88	—	100	336	1	100	237	—	77	139	—	75	78	—	76	100	—
Scoloplos armiger	13	4	—	25	6	—	40	4	—	14	1	—	28	3	—	30	4	—	53	8	—
***Ophelia* sp.**	—	—	—	56	13	—	40	6	—	—	—	—	22	3	—	40	6	—	95	40	2
Travisia forbesii	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	24	3	3
Ammotrypane aulogaster	—	—	—	—	—	—	—	—	—	—	—	—	6	1	—	5	1	1	5	—	—
Cirratulus spp.	100	61	2	94	100	6	90	141	9	100	146	3	100	218	13	95	161	12	95	262	28
Euchone analis	50	10	—	25	6	—	20	8	—	19	10	—	22	5	—	30	6	—	43	14	1
Other sedentary polychaetes	38	26	—	81	97	3	70	113	2	86	47	1	83	45	1	75	51	1	81	101	4
Other unidentified worms	63	30	—	44	11	—	10	6	—	29	46	—	39	12	—	35	7	—	29	4	—
Anonyx nugax	13	1	—	31	5	—	50	7	—	29	6	—	28	4	1	30	7	—	76	36	1
Other amphipods	100	79	—	94	63	—	100	177	2	100	124	1	77	59	—	90	99	1	90	435	5
Nebalia bipes	75	133	1	94	92	1	90	127	1	100	70	—	66	45	—	90	96	1	86	68	—
Axinopsis orbiculata	88	68	1	75	88	1	80	84	1	71	149	1	94	123	1	90	80	1	90	57	1
Serripes groenlandicum	—	—	—	19	2	30	10	1	1	—	—	—	28	3	1	20	2	10	10	1	—
Macoma calcarea	100	211	21	94	279	48	90	481	62	100	587	31	100	416	42	95	442	24	100	363	46
Mya truncata	63	30	—	94	78	4	80	249	6	100	144	2	94	31	2	85	90	3	100	114	11
***Thracia* spp.**	—	—	—	25	4	1	—	—	—	—	—	—	6	1	—	15	2	—	67	19	2
Other lamellibranchs	—	—	—	—	—	—	20	2	—	14	1	—	—	—	—	—	—	—	—	—	—
Diaphana minuta	—	—	—	6	1	—	10	1	—	—	—	—	17	2	—	20	2	—	24	3	—
***Cingula* spp.**	50	24	—	50	32	—	90	145	1	100	81	—	39	11	—	70	34	—	81	260	1
***Buccinum* spp.**	—	—	—	6	1	—	10	1	—	—	—	—	—	—	—	—	—	—	—	—	—
***Bela* spp.**	38	8	—	63	9	—	60	11	—	57	26	1	72	14	—	70	16	1	67	14	—
Other gastropods	—	—	—	—	—	—	10	1	—	14	1	—	—	—	—	—	—	—	—	—	—
Ophiuroids	—	—	—	13	1	—	—	—	—	—	—	—	11	1	—	—	—	—	—	—	—
Cucumaria calcigera	—	—	—	—	—	—	—	—	—	—	—	—	6	1	—	15	2	1	5	—	—
Other holothurians	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5	—	—
Totals		958	37		1,132	105		2,105	99		1,879	49		1,358	76		1,377	78		2,174	130

МОРСКОЙ БЕНТОС (ИНФАУНА) АРКТИЧЕСКОЙ СЕВЕРНОЙ АМЕРИКИ

Количественные исследования морского бентоса (инфауны) был произведены в Арктик Бей и Понд Инлет (северная Баффинова Зем ля) в 1954 и 1955 гг., в Годхааб и Годхавн (западная Гренландия) 1956, и в Фрустрейшен Бей (Фокс Бассейн) в 1957 г.

Явно выраженные *Macoma*-сообщества с биомассой обычно выш 200 грм. на 1 м². (живой вес) были встречены на мелководьи, на глу бине до 50 м., в большинстве исследованных участков. Сообществ *Chiridothea sabini* было найдено на мелководьи в Фрустрейшен Бей Foraminifera-сообщества с биомассой обычно меньше 100 грм. на 1 м² были обнаружены на глубине свыше 50 м. в северной части Баффи новой Земли, а также в заливе Диско.

Биомасса бентоса, выраженная в весе сухого органического ве щества, была обычно 15 грм., или больше, на 1 м². на глубинах д 50 м., и 10 грм., или меньше, на 1 м². на бóльших глубинах.

Эти исследования, равно как и произведенные раньше в Гренлан дии, показывают, что *Macoma*-сообщества встречаются во многи местах в арктической северной Америке, но лишь там, где темпера тура морской воды, постоянно или сезонно, ниже-арктическая, то-ест 0—5°С. На крайнем севере эти сообщества оказываются приурочен ными к мелководьям, нагреваемым летней инсоляцией до нижне-арк тического уровня. Сообщества *Gomphina fluctuosa* и *Portlandia arctica* нижне-арктических условиях среды приурочены повидимому к опреде ленным типам данных отложений: крупный песок для первого сооб щества и тонкий ил для второго. Сообщества *Chiridothea sabini* был найдены только на мелководьи в двух местах, оба повидимому постоянной верхне-арктической температурой, то-есть ниже 0°С. Со общества foraminifera и *Astarte crenata* были найдены исключительно на глубинах вне влияния инсоляции, где температурные условия мо гут быть либо нижне- либо верхне-арктические.

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A HYPOCHOLESTEROLEMIC FACTOR IN MARINE STEROLS

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During recent years, several previously undescribed sterols have been isolated from marine invertebrates in this laboratory. 24-Methylenecholesterol was identified as the principal sterol of the butter clam (*Saxidomus giganteus*) (1) and a major component of several other edible molluscs (2). Other sterols of the clam have not been identified but evidence has been presented indicating that cholesterol is not a significant component (1, 2).

We wish to report that a sterol mixture of the butter clam exerts a hypocholesterolemic effect in chicks.

Previously, such effects have been observed following controlled feeding of cholestanol (3), β-sitosterol, ergosterol, or stigmasterol (4), and from a substance found in lingcod liver oil (5, 6).

The mixture of clam sterols was isolated according to published procedures (1, 2). The sterols were purified with charcoal and one recrystallization from ethanol.

Groups of sixteen 8-day-old chicks were maintained on either a standard diet (5), a standard diet supplemented with 1% cholesterol, or a standard diet supplemented with 1% cholesterol and 1% clam sterol mixture.

On the 14th day of test feeding, blood samples (1.25 ml) were taken from the brachial vein and pooled in lots of four prior to collection of serum. Serum cholesterol levels were measured according to the method of Sperry and Webb (7). The results are summarized in Table I.

TABLE I

Effect of diet on serum cholesterol levels

	Basic diet	Basic + 1% cholesterol	Basic + 1% cholesterol + 1% clam sterol
	Serum cholesterol* (mg%)		
	135	585	262
	192	707	354
	109	581	301
	191	638	507
Mean ± S.E.	157 ± 21	628 ± 30	356 ± 54
Food consumption†	17.7	17.0	17.1
Weight gain‡	95.4	89.1	88.1

* Analysis of variance: groups 2 and 3, $P = 0.01$–0.001; groups 1 and 2, $P = <0.001$; groups 1 and 3, $P = <0.001$.
†Grams/chick/day.
‡Grams/chick.

Feeding experiments, using synthetic 24-methylenecholesterol (8) will be the subject of a future communication.

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Are the Pinnipedia Biphyletic?

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Are the Pinnipedia Biphyletic?

I. A. McLAREN

WHILE more primitive ancestors (creodonts, miacid carnivores) have been suggested in the older literature, respectable anatomical opinion seems to assure us that the Pinnipedia are derived from canoid (*sensu* Simpson, 1945) carnivores. This paper will support the generally discredited view that the fur seals, sea lions, and walruses, together, have a terrestrial ancestry separate from the hair seals.

The district anatomical differences between the Otariidae and Odobenidae on the one hand and the Phocidae on the other have been emphasized by some authors and belittled by others, in the light of the essentially common plan and mode of life of the Pinnipedia. There is little merit in citing anatomical differences alone as evidence of the dual origin of a taxon. The even more striking differences between the two suborders of Cetacea have in effect simply been listed (most recently by Kleinenberg, 1958) in favor of a biphyletic origin, but the argument is unfinished without evidence of suitable ancestors outside the primitive archaeocete whales.

Several authors, of whom Mivart (1885) seems to have been the first, have suggested on anatomical evidence that the otariids and walruses are derived from bear-like ancestors and the phocids from otter-like stock. Some reviewers (e.g., Kellogg, 1922; Howell, 1929, 1930; Simpson, 1945) have not felt able to exclude entirely the possibility of biphyletic origin, while others (Winge, 1941; Weber, 1904, 1928) have adamantly dismissed the notion. Simpson (1945) offers perhaps the most concise reconciliation of these otter-like and bear-like traits with the idea of monophyletic origin: "Probably the pinnipeds are an early offshot of the little differentiated late Eocene and early Oligocene canoid ancestry, paralleled by the otters, which had the same ultimate origin and a similar adaptive trend, and in other features by the bears, also with the same remote origin." It is believed, however, that some of the support for a monophyletic origin involves errors of reasoning, and that evidence has accumulated in recent years which makes a biphyletic origin from otter-like and bear-like ancestors more likely.

Anatomical Evidence from Recent Forms

Weber (1904) believed that the soft-part anatomy of pinnipeds indicated a relationship with bears. However, as pointed out by Kellogg (1922), the anatomical features used by Weber do not support his contention—being either too variable (intestinal flexures), related to body size (lobulated kidney, also in otters), simply reflecting the general or primitive carnivore condition (bicornate uterus, endotheliochorial and zonary placenta), or indicating specifically canoid rather than feloid relationships (absence of Cowper's gland).

Other soft-part anatomy seems to support a carnivore or, when specific, a canoid derivation, but does not suggest closer affinity with one or other canoid family. A reduced caecum is present in the pinniped intestine, although lost in other Canoidea except Canidae (Mitchell, 1916). Fish (1903) lists several features in common in the superficial anatomy of the brain of *Ursus* and some pinnipeds; the resemblances are somewhat less marked between the bear and the Phocidae. The "ursid lozenge" of the brain is typical of Ursidae, Mustelidae, and several pinnipeds. However, Jelgersma (cited in Mohr, 1952) studied exhaustively the microscopic and macroscopic anatomy of the

brain structure of several aquatic mammals, and concluded that *Phoca* is derivable from terrestrial carnivores without specifying what these carnivores might have been. The placental labyrinth of pinnipeds is lobulated, as in Canoidea but not Feloidea (Rau, 1925). The endothelium of the maternal capillaries is much thicker in the placenta of Mustelidae than in that of the Ursidae and Canidae (Amoroso, 1952); unfortunately there appears to be no published information on this condition in the pinniped placenta.

In spite of marked differences in the skeleton and body musculature of the Otariidae and Phocidae, each group is in its own way highly aquatically adapted, and possible anatomical links with separate living carnivores are thus obscured. One bone which cannot have been aquatically adapted is the os penis, and it is distinctly canoid rather than feloid in structure.

Differences between the skulls of Phocidae and Otariidae were the basis of Mivart's (1885) original argument in support of a biphyletic origin. Some of these differences are trivial and will not be repeated here, but the conservative basicranial region of the skull has become accepted as important in the classification of Carnivores, and this region is certainly bear-like in the Otariidae and otter-like in the Phocidae:

1) In the Phocidae and Mustelidae there is no alisphenoid canal, while in Otariidae, Odobenidae, Ursidae, Canidae, and most Procyonidae it is present.

2) The bulla of *Lutra* could easily be made to resemble that of *Phoca* by giving a rounded form to the mastoid; in both genera there is the same sort of groove between the mastoid and the tympanic. The bulla of the Otariidae, on the contrary, is very like that of the Ursidae, and the sort of groove found between the mastoid and tympanic of *Lutra* and *Phoca* is absent.

3) The mastoid process hangs ventrally considerably in the Ursidae and Otariidae, but not in Phocidae and *Lutra*.

4) In the Phocidae and Lutrinae the paroccipital and mastoid processes are not united by a ridge of bone, while in Ursidae and Otariidae they are so united.

5) More generally, the light crania of *Lutra* and the most primitive hair seals (Phocinae) have noteworthy defects in ossification of the walls. The massive skulls of Ursidae, Otariidae, and Odobenidae have few or no such defects.

Van Kampen (1905) noted these basicranial similarities, but found that ossification of the endotympanic may be involved in the bulla—as in Feloidea but not in modern Canoidea. A separate endotympanic element was described in a foetus of *Cystophora* (a highly specialized phocid genus), and a suture was noted on a bulla of a foetal sea lion, *Otaria*, apparently dividing the tympanic from the endotympanic part. The endotympanic was imputed to other pinnipeds on the basis of grooves on the bullae, often quite indistinct, although indistinct grooves are known on some canoids as well. In the pinnipeds, as in the Canoidea, there is no septum bullae, which demarcates the endotympanic and tympanic in Feloidea. Van der Klaauw (1931) states that the endotympanic of other Phocidae begins development in cartilage, as in modern Canidae, and that incipient ossification may occur in some Canidae and the badger *Meles*. It may be that ossification of the endotympanic in at least some pinnipeds is a secondary developmental approach, or due to the retention of the ossified condition known in fossil canoids. Subsequent authors vary in the amount of phylogenetic significance they attach to Van Kampen's work on pinniped bullae, but the present author believes it is unwise to dismiss the resemblances of the basicranial region to the same region in Ursidae and Mustelidae, when other anatomical evidence points to the canoid affinities of the pinnipeds.

There has been a tendency in the litera-

ture to accept the resemblances between the Otariidae and Ursidae, but not those between Phocidae and Lutrinae. The most recent relevant work of which the author is aware (Dornesco and Marcoci, 1958) is perhaps typical. They conclude that the skull of the "Pinnipedia" (by which they mean three species of hair seals) when compared in detail with the skulls of *Canis, Felis, Ursus, Meles,* and *Lutra,* most resembles the last. Faced with suggestions in the literature of derivation from undifferentiated canoid carnivores or amphicyonids, they conclude that the resemblances are convergent, produced by adaptation to aquatic life. It is difficult to understand how aquatic adaptation could thus affect the basicranial region, and it should be pointed out that the resemblances of the skull of Phocidae to that of the otter are most marked in the smaller species of the northern seal subfamily Phocinae; these same Phocinae are certainly anatomically the most primitive and *least* aquatically adapted of the living hair seals (see Laws, 1959).

On the other hand, to Winge (1941) "it hardly can be doubted that both these groups spring from the same ursid stock," and Weber (1928), who abandoned his earlier (1904) belief in ursid origins, substituted pre-ursids (perhaps amphicyonids) instead. If we examine their arguments we find that they rest on the assumption, widely held, that the Phocidae are more aquatically adapted and the Otariidae more primitive. Thus the Otariidae are presumed to resemble more closely their terrestrial ancestors and contrary characters in the Phocidae can be explained as convergent; but it must be noted that this argument involves the *a priori* assumption that the Pinnipedia are monophyletic.

The Problems of Time

One of the chief objections to a biphyletic origin has been the belief that the pinnipeds must have arisen from early undifferentiated Canoidea, antedating the origin of the ursid and lutrine lines. A recent enquiry into pinniped zoogeography (Davies, 1958) suggests that pinnipeds must have originated between middle Eocene and middle Oligocene, and favors the late Eocene as most likely.

The ages of early fossil pinnipeds have consistently been overestimated. Actually, on revisions of European stratigraphy, the earliest phocid seal (apart from unacceptable teeth or fragments) is the humerus named *Leptophoca lenis* by True (1906). This was found in the Calvert formation in Maryland, which is listed as lower middle Miocene (but may be younger, as the Helvetian, its European correlate, is now thought to be upper middle or lower upper Miocene). The earliest fossil Otariidae of the west coast of the United States, which have been listed as early Miocene by Kellogg (1922) and others since, are now best considered to be late middle Miocene (Downs, 1953).

The earliest remains are of highly differentiated seals. Several genera of Otariidae are described, and among the phocid seals all the present subfamilies are known and there is a representative of the modern subgenus *Pusa* from the Mio-Pliocene (McLaren, 1960). This early specialization has been supposed by many authors to reflect a long prior evolution. However, as Downs (1956) has pointed out, the shift from one adaptive zone (land) to another (water) is likely to be accomplished by very rapid, "quantum" evolution.

As the seals can be shown to have appeared later and to have evolved more rapidly than is generally supposed, so can suitable biphyletic ancestors be demonstrated farther back in the record.

The Mustelidae can be traced to the late Eocene. More important, aquatic mustelids, in fact the primitive otter *Potamotherium,* often formerly listed as Miocene, are known as early as Stampian or middle Oligocene. Savage (1957) believes that we must look for the beginnings of the Lutrinae in lower Oligocene.

The Ursidae are the last of the canoid families to appear. Possible bear ancestors are described from the Miocene, but undisputed bears are unknown before the Pliocene. However, it would be naive to insist upon adapted Ursidae as ancestral to the Otariidae. The Canidae seem to have resolved themselves from the dog-bear stock in the Oligocene, and bear-like traits were expressed in some forms at that time (see Hough, 1948).

Thus in summary we have something of the order of at least ten million years for the derivation of the first fossil otariids and phocids from possible separate ancestral stocks. This might be quite sufficient for the "quantum" evolution envisaged by Downs.

The Problems of Parasites

Parasites, which are sometimes more conservative than their hosts, can give valuable clues to phylogenetic history. The internal parasites of seals do not seem to give evidence of pinniped origins, perhaps because many are vectored by alternate marine hosts. The otariids and walruses have one genus of halarachnid mites and the phocids another (Newell, 1947). The anoplurid lice, however, have been suggested as having important phylogenetic significance by Hopkins (1949) and must be considered here.

The Anoplura of seals belong to the family Haematopinidae, elsewhere found only on the Canidae. Hopkins believes members of this louse family to have been parasites on the primitive carnivores. There is no need to explain their survival in pinnipeds, where mallophagan lice have become extinct, for if they were able to survive the aquatic existence they would be free from competitive extinction brought about by contact with evolved lice on terrestrial carnivores. However, the Haematopinidae from otariids, walruses, and phocids constitute a distinct group, quite different from their nearest relatives on the Canidae. Hopkins adduces this as evidence for the monophyletic origin of the Pinnipedia. It is possible that primitive lice survived true-to-type but separately in the two groups of seals, while they were modified from the primitive type on the Canidae, but a more telling argument against Hopkin's conclusions is found in his own data. The most primitive genus of lice has been found only on the northern fur seal. Another genus is confined to the southern elephant seal. A third genus, *Echinophthirus,* has been found only on seven species of northern-hemisphere hair seals. Most significantly, the fourth genus, *Antarctophthirus,* is found on five species of Otariidae, the walrus *and* the four species of Antarctic hair seals. We can only safely conclude that if *Antarctophthirus* has shown obvious cross-infestivity between otariids and phocids, then interchange of parasites was likewise possible in the past, and we cannot be sure that this peculiar group of lice was present in the ancestors of all the pinnipeds.

The Problems of Zoogeography

Davies (1958) has given the most recent and best account of the early history of the pinnipeds, presuming them to be monophyletic. However, his discussion appears to the present author to contain anachronisms and zoogeographical difficulties which can be resolved by presuming a dual origin of the pinnipeds.

Davies holds that the seals originated in the Arctic basin and have been confined to cool waters throughout their early history. The problem then is to derive two highly divergent groups from this common Arctic stock. The entire early history of the Otariidae was certainly confined to the North Pacific and the early hair seals are all found in the Atlantic-Tethyan region. Davies invokes the Bering Sea barrier, on evidence from Simpson (1947), as responsible for the divergencies in the pinniped stock. While he does not feel that movement at other times can be excluded, he concludes that "the middle Eocene, the middle to late Oligocene, and

perhaps the early Pliocene were times of exceptionally easy movement between the Arctic and the North Pacific."

Davies supposes that the break of the barrier in the middle Eocene would have been that required for the spread of the original population between Arctic and Pacific, and the subsequent closure of the barrier would allow divergence of the two forms. Present-day seals (except the tropical *Monachus* and certain otariids) are confined to waters cooler than 20°C., and Davies suggests that climatic restrictions similarly prevented early seals from venturing outside northern seas to leave their remains in presently accessible deposits until middle and late Miocene.

The later opening from middle to late Oligocene (the bridge probably did not close strongly until middle Miocene) is used by Davies to allow the ancestors of the walruses, obviously derived from otariid stock, to re-enter the Arctic seas. There they developed their special mode of life in isolation, appearing as *Prorosmarus* in the upper Miocene of eastern North America and as essentially modern walruses in the Pliocene of western Europe. An earlier suggestion by Kellogg (1922) that the walruses invaded the Atlantic by crossing submerged Central America is climatically unlikely.

There are two major difficulties with this analysis:

1) Since the development of the canoid stock (which Davies accepts as ancestors) did not occur until late Eocene and early Oligocene, it would seem that the middle Eocene is somewhat early for the Arctic origin of the pinnipeds and their movement through the Bering Strait. Although such datings cannot be taken as exact, Davies himself seems to have sensed the anachronism, for he suggests the late Eocene as the most probable time of origin elsewhere in his paper and states that his zoogeographical analysis "would necessitate a slightly earlier origin for the pinnipeds than favored by most."

2) Since to give rise to the walruses the evolved otariid stock must certainly be assumed to have entered the Arctic from the North Pacific some time between the middle Oligocene and, at the latest, middle Miocene, why did not the evolved hair seals in their turn invade the North Pacific at that time? Of course we may invoke many special arguments, but they must account for the fact that the hair seals *did* enter the Pacific from the north in the Plio-Pleistocene. It seems most logical to assume that the hair seals did not exist in the Arctic Ocean in the vicinity of the Bering Strait in the Oligocene or Miocene, although their ancestors must, on Davies' arguments, have been there earlier.

Accepting the separate geographical distributions of the earliest otariid and phocid seals, we may add further evidence that this separation was primary, and due to their biphyletic origin.

Apart from the single occurrence of *Leptophoca lenis* in eastern North America (mentioned earlier) all the earliest Phocidae are found in southern and western Europe (see Fig. 5 in McLaren, 1960). It is difficult to explain this predominance solely in terms of climate or accessibility of deposits. There seems to have been an especial favoring of the Tethyan region in the early fossil record, suggestive of an early evolutionary radiation in that area. While we must recognize the limitations of this sort of evidence, it can be pointed out that probably the earliest mustelids and certainly the earliest known otters were Palearctic.

Similarly, to match the appearance of the otariids first on the Pacific coast of the United States, we find the Oligocene elaboration of the dog-bear stock in North America (see Hough, 1948).

The Significance of Carnivore Serology

Leone and Wiens (1956) examined the serological relationships between representatives of all the families of Carnivora. They found that the reactivity of fur-seal antiserum was greater with ursid serum

than with that of any other family. Other reactions suggested the Canidae to be next closest serologically. The cross-reactivity of ursid sera was greater than that of any other canoid family, and Leone and Wiens suggest that the bears are a conservative and biochemically undifferentiated line, more representative of the early canoid stock. They conclude that the Pinnipedia are to be derived from canoid carnivores (already forecast on anatomical grounds) and that they do not deserve ordinal or even subordinal rank, but are to be included under Canoidea.

These are important results, but they have one limitation: the serological reactions of the Phocidae were not examined, for the only representative of the pinnipeds was a fur seal, *Callorhinus ursinus*. Thus we can only say that the Otariidae are most closely related to the Ursidae, and that this may indicate a direct descendence from primitive ursid stock, or may reflect a common serological conservatism from primitive dog-bear ancestors. The Mustelidae are excluded from this phylogeny, and we cannot presume that the Phocidae are involved.

Potamotherium and Semantor

Kellogg (1922) drew attention to *Potamotherium valletoni* Geoffroy, 1833, of Tertiary lacustrine deposits of Europe, which "presents many characters which may illustrate relationship with the Phocidae." The skeletal characters are of course in the direction of aquatic adaptation, but Savage (1957) in his extremely careful and thorough appraisal of the anatomy of *Potamotherium* believes the resemblances to *Phoca* to be of real phylogenetic significance. The skull of *Potamotherium* is lutrine, as the jaws and teeth are adapted to mastication. In one feature, however, the skull answers an objection offered by Mivart (1885) to his proposal of phocid-lutrine relationships: the skulls of *Lutra*, Ursidae, Otariidae, and indeed almost all carnivores, have strongly expressed postorbital processes on the frontals, but these processes are absent on the Phocidae and vestigial in *Potamotherium*.

Orlov (1933) described in great detail the hind half of a very peculiar animal discovered in late Tertiary deposits of West Siberia. It showed features of both otters and seals, and Orlov, believing that resemblances with the latter could not be convergent, named the animal *Semantor macrurus*, as the sole representative of a new family of pinnipeds, the Semantoridae. Thenius (1949) decided from the data of Orlov that of eighteen parts of the skeleton, eleven were essentially lutrine, six intermediate, and one seal-like. He then concluded that *Semantor* was no more than an aquatically highly adapted otter, and its resemblances to hair seals a result of convergence. Recently Kirpichnikov (1955) has pointed out the dangers of treating unweighted anatomical characters in this way, and has further resurrected the importance of *Semantor* by describing the humerus, perhaps from the same individual described earlier by Orlov. This humerus is very like that of the hair seals and much less like that of *Lutra* (although considerably more like the humeri of the sea otter *Enhydra* and of *Potamotherium*). To Kirpichnikov the anatomy of *Semantor* not only bespeaks a relationship with the pinnipeds, but he goes so far as to suggest that resemblances between the axial skeleton of *Semantor* and otters are superficial, and not reliable indications of phylogenetic affinity.

In view of these divergent ideas of the significance of *Semantor*, its characteristics are repeated here in a general way. There is no doubt that the axial skeleton is essentially lutrine. Except for the larger size of *Semantor*, its sacrum, pelvis, and femur are very like those of *Potamotherium*, in which, of course, Savage (1957) has shown these bones to approach the pinniped condition in several respects. The tibia and fibula are fused proximally, as in pinnipeds but not lutrines. Although the hind limb could probably be brought

forward, as in the fissipeds and Otariidae (the lesser trochanter of the femur, although reduced, was not lost as in Phocidae), the ankle approached the phocid condition in its mobility, and the foot could probably be rotated somewhat around the axis of the shank. The astragalus, although lacking the calcanear process typical of Phocidae, had lost the groove of the trochlea; this groove is well developed on fissipeds and Otariidae, but is lost in Phocidae.

In summary, one must agree with Savage (1957) that "while *Potamotherium* is a morphological link between *Lutra* and *Phoca, Semantor* is a morphological link between *Potamotherium* and *Phoca.*" Of course any such morphological series can be explained by convergence, as Thenius (1949) has done. But if we accept this as an explanation of the conformities of *Semantor* and Phocidae, then we are obliged to acknowledge the possibility of comparable convergence between the Otariidae and Phocidae. In certain critical ways *Semantor* resembles the Phocidae more than do the Otariidae, and the skeletal similarities of the two types of seals are certainly all connected with their higher degree of aquatic adaptation. *Semantor* is found in imprecisely dated deposits, but Kirpichnikov (1955) refers it to upper Miocene or lower Pliocene—too late to be directly ancestral to the hair seals. It also had at least one specialized feature, in the fusion of entocuneiform and mesocuneiform, unknown in seals or otters. Nevertheless, one must agree with Orlov that *Semantor* is a striking confirmation of Mivart's (1885) belief that the hair seals were derived from otter-like forms.

It is important to note that there is no room for the Otariidae in any relationship between the Phocidae and putative lutrine ancestors. If the otariids are related, their anatomy and serology do not suggest it. Although Howell (1930) believes that the otariid limbs in some respects show greater aquatic adaptation than the phocid, most authors give convincing evidence that the otariids are in many respects less emancipated from their terrestrial morphology. If the hair seals are derived from lutrine stock, then the Pinnipedia cannot be monophyletic.

A Speculative Account of Seal Origins

The author disclaims any originality in the preceding sections. Every suggestion made has its counterpart in the literature, and several authors have been attracted to the idea of biphyletic origins. However, it is believed that all the evidence together constitutes a more powerful argument than has hitherto been presented, and justifies a speculative enquiry beyond the limitations of present evidence.

Central Asia has been characterized since the late Mesozoic by the development of a vast freshwater system. The extent and permanence of this system has offered opportunities for aquatic evolution elsewhere matched only in the seas. The seemingly marine facies, diversity, and endemism of the fauna of Lake Baikal must owe much to this earlier and larger system, of which this great lake is one of the last vestiges (Martinson, 1958). The extensive and deep basins of the Mesozoic began to break up in the early Tertiary, but fresh water was widely persistent, and warmth-loving faunas flourished. In the late Tertiary, marine transgressions had their effect and the present fauna of the Caspian Sea derives much from that time.

It is significant that only in this part of the world was there much likelihood of development of a truly aquatic mammal. *Potamotherium* flourished in the Aquitanian lakes of the late Oligocene of France. Early Miocene orogeny destroyed the lakes and *Potamotherium,* but its relatives continued to evolve beyond the amphibious stage in the permanent lakes of Asia to the east.

Semantor must be considered an important link in the evolutionary series. The unknown skull of *Semantor* would determine finally whether this animal

should be classed as an otter or a hair seal. It was undoubtedly much more competent in the water and probably more piscivorous than present-day freshwater lutrines. It was also larger, and therefore probably more capable of swallowing its prey whole. One might predict that the teeth had lost their masticatory function and were adapted to seizing and holding fish; such secondary homodonty would surely make *Semantor* a primitive hair seal. The fate of *Semantor* was extinction by disruption of the freshwater habitat and perhaps climatic deterioration in the late Tertiary. Its relatives, the even more aquatic Phocidae, were able to take advantage of Miocene marine transgressions and invade the seas.

There are some reasons to suggest that the first true hair seals (considering the systematic position of the Semantoridae to be debatable) were inhabitants of the great inland waters of Asia. The most primitive Phocidae, the subgenera *Phoca* and *Pusa*, show a strong predilection for fresh water (the Baikal and Caspian seals and the freshwater races of the ringed and harbor seals). A freshwater origin in the Palearctic would also help explain the almost simultaneous appearance in the fossil record of southeast Europe of seals belonging to the ice-breeding northern group (Phocinae) and the tropical seals (Monachinae). We may imagine that the northern freshwater seals, perhaps by now cut off from the south, adapted to the increasing cold of the Miocene and developed the ice-breeding habit in the innermost continental reaches of the lacustrine system, or perhaps in marine transgressions from the Arctic Ocean. The ringed seal group of Lake Baikal, the Caspian and Holarctic seas moved into southeastern Europe, almost certainly from the north, at the end of the Miocene (McLaren, 1960), and is probably quite close to this primary freshwater stock. Seals in the southern part of Asia, responding to warm climates and marine transgressions, became the Monachinae of the Tethyan-Mediterranean area, whose eurythermy permitted them to cross the tropics to the Antarctic and give rise to the four related species of hair seals there, perhaps during the cooling of the late Miocene (Davies, 1958).

We cannot offer any ancestor for the otariid seals as well delineated as the ancestral Phocidae. We may presume that they were members of the same early dog-bear stock which has been suggested as ancestral to all the pinnipeds.

Davies (1958) has argued that the Pacific coast now and throughout the Tertiary with its steep, surf-battered shores and folding parallel to the coast was an inauspicious place for pinniped ancestry. In fact, as pointed out by Scheffer (1958), the North Pacific with its protected, food-rich kelp reefs is a very suitable place, and offers the sea otter as an example of more recent marine invasion. The North Pacific, where the first fossil otariids are found, was colonized not from another ocean but from its own shores, probably from northwest North America in the Oligocene.

When the Phocidae entered the sea they were already highly adapted lacustrine carnivores, as sleek and efficient as we know them today. It is perhaps significant that the otters are inclined to be fossorial. Savage (1957) indicates that even in *Potamotherium* the forelimb may have been used in digging. (Are the primitive ringed and Baikal seals merely preadapted to digging out their birth lairs in the snow on the ice, or were the ancestral Phocidae fossorial?) Thus the Lutrinae and by inheritance the Phocidae have concentrated on the hind limb as an organ of propulsion.

The Otariidae, on the other hand, entered the sea across the northwest coast after a history as littoral carnivores. Their evolution was rapid and their aquatic adaptation very thorough, but still a modification of a terrestrial morphology rather than an improvement on an already established aquatic morphology. The otariids

have concentrated on developing the forelimb—a great improvement on the primitive "dog-paddle." Most otariids spend much time on land in large social units, and retain much more terrestrial competence than the Phocidae. This was permissible among the skerries and reefs of the North Pacific, with the freedom from terrestrial predation. The more primitive Phocidae tend to avoid the land (the ringed seal almost never comes out on land in the ice-free summer, and the harbor seal is a wary animal of inaccessible banks and skerries). Extremes of sociality and the tendency to spend much time on land have developed among the higher Phocidae (see Laws, 1959). Both the land habit and gregariousness were unsuitable in inland lakes and seas, with the danger from terrestrial predation.

We will not conclude by offering a taxonomic revision of the pinnipeds as a group of biphyletic origin. It is believed that the burden of proof now lies with those who assume a monophyletic origin. Before we can speak with any finality, more fossil evidence will be required. As a final statement the author would like to plead that the entire carnivore series be examined again serologically, this time including both phocid and otariid seals in the analysis.

Summary

1. Anatomical evidence suggests that pinnipeds are derived from canoid carnivores. Mivart (1885) was first to point out that the conservative basicranial regions support a derivation of Otariidae (and Odobenidae) from bear-like and Phocidae from otter-like ancestors. Possible differences from modern canoids in the development of the pinniped bulla do not appear to discredit these relationships. There has been a tendency to accept the connection of Otariidae with Ursidae, or pre-ursids, and to reject the phocid-lutrine resemblances as convergent, but the reasons for this seem invalid.

2. It has been objected that the pinnipeds must have appeared before the evolution of the ursid and lutrine lines of carnivores. The first fossil Phocidae appear in lower middle and the first Otariidae in upper middle Miocene. The Lutrinae probably originated in early Oligocene and suitable otariid ancestors could be found among the dog-bear stocks of that time. Aquatic mammals can be expected to develop by "quantum" evolution, and at least ten million years are available for the biphyletic evolution of pinnipeds before their appearance as fossils.

3. The anoplurid lice of Pinnipedia have been used to support a monophyletic origin, but the argument is shown to be untenable.

4. The paleozoogeography of pinnipeds indicates that the Otariidae developed in the North Pacific and the Phocidae in the Palearctic, and that their early geographical separation was primary, and due to separate origins.

5. Serological study of the carnivores has been taken as added evidence that the pinnipeds are closely related to Ursidae and probably derived from undifferentiated Canoidea; however, only an otariid seal represented the Pinnipedia in this study.

6. The late Oligocene otter *Potamotherium* has many anatomical foreshadowings of the Phocidae. Orlov (1933) described *Semantor macrurus* from the late Tertiary of West Siberia as representing a new family of Pinnipedia. The axial skeleton is essentially lutrine, the hind limbs and girdle intermediate, and the hind pes and humerus are markedly phocid-like. Such adaptations to aquatic life can always be dismissed as convergent, and the systematic position of *Semantor*—whether otter or seal—has been debated, but there appears little doubt that *Semantor* is a valid morphological link and may be a descendent of the primitive hair seal ancestors.

7. It is suggested that the Phocidae arose from lutrine ancestors in the extensive and permanent lacustrine systems of

Tertiary Asia, and that they invaded the seas through marine transgressions in the Miocene. The Otariidae may have developed from littoral canoid carnivores of the northwest coast of North America. These separate origins seem to account for certain fundamentally divergent anatomical and behavioral traits in the two kinds of seals.

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Biochemical Changes in Fish During Maturation

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Biochemical Changes in Fish During Maturation

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It has long been recognized that marked changes occur in many species of fish during the normal sexual cycle, but, in comparison with mere observation, actual chemical studies of these changes have not been plentiful. In general, there is a strong tendency to build up large energy reserves, particularly of fat, during the active feeding period prior to spawning. These fat reserves, which are usually termed "depot fats," are depleted during the comparatively rapid formation of milt and roe. These are the gross changes, and of course the picture will differ with fish species. Indeed, with the cartilaginous and viviparous dogfish, which bear their young in the winter months, the amount of liver oil does not seem to vary much with sexual condition. Changes in proteins and other nitrogenous constituents have also been observed, and it is my purpose to review some of the interesting work which has been carried out by widely separated investigators on different species. As a preliminary it is necessary to draw attention to some of the important chemical constituents of fish. These may be listed roughly as follows, and of course it must be realized that there are considerable variations with species and, as will be later noted, with sexual maturity.

Proteins.

Practically all studies of fish proteins have been concerned with muscle, and little is known regarding proteins of most other important tissues. The muscle proteins may be roughly classified into three categories: (1) Proteins extracted by neutral salt solutions of rather high ionic strength. Actomyosin, the important "contractile protein" combination, accounts for about 65% of muscle protein. Other minor proteins such as tropomyosins are also present. (2) Proteins extractable by neutral salt solutions of comparatively low ionic strength. There are usually about 8 to 10 proteins separable by physical techniques (electrophoresis; ultracentrifuge). This division is arbitrary and it is probable that there may be a very much larger number of proteins. There are definite variations in different classes of fish. Many of the muscle enzymes are present in this fraction. (3) Non-protein nitrogen. This fraction accounts for from 9.2% to 18.3% of the total muscle nitrogen in teleost fish and 33% to 38.6% in elasmobranch fish. It consists of a large number of comparatively low molecular weight substances; volatile bases such as trimethylamine and their precursors, particularly trimethylamine oxide; guanidine derivatives (creatine, creatinine and arginine); imidazole or glyoxaline derivatives (histidine, anserine, carnosine) and amino acids, urea and purines. (4) Stroma proteins; collagens and elastin.

Lipids.

The lipids of fish represent an important and very variable general constituent. Roughly they may be classed as the true triglyceride fats, i.e., three fatty acids esterified with one molecule of glycerol. The fatty acids are usually complex, and there is a considerable proportion of long-chain polyunsaturated acids. Also, there are a number of phospholipids which have, in general, not been the subject of intensive study except in isolated instances. They are probably associated with the lipids of "constitution" rather than with so-called "depot" lipids or fats. In some non-fatty fish such as cod they represent an important fraction of the total muscle lipid material. Compounds such as lecithins, choline, phosphatidic acids, inositol lipids, serine lipids, etc. are representative of this class of substances. In addition, and apart from the blood, there are many other compounds which may only account for a minute fraction of the total solids in fish, but may exert very important biochemical functions. Thus cholesterol (free and esterified), inorganic salts, nucleic acids and nucleotides, pentose and hexose phosphate esters, glycogen, steroid hormones, etc. undoubtedly play an important role in development and maturation of fish. Little is really known about the occurrence, distribution and functions of many of these compounds in fish and it is hoped that in the future biochemists, physiologists, and organic chemists will pay more attention to fish, and that biologists and zoologists may help them in their studies.

For convenience, this presentation has been divided into two parts, namely, (1) general investigations, and (2) studies on migrating salmon.

General Investigations

PROTEINS

Recently, useful contributions to our knowledge of changes in cod which occur with increasing age and season were made (Love, 1958). Work was original in respect to the fact that the muscle myotomes were physically separated from the septae or myocommata before making many of the analyses. It has often been stated that about 90% of cod muscle protein is soluble in 5% sodium chloride solution, but this value has been disputed to some extent. The above work showed that in whole fillets of North Sea cod the amount of soluble protein depended on the size of the fish. Thus in small fish (about 12 inches) approximately 87% was soluble, while in 34-inch fish only 68% dissolved. It was found that the amount of protein in the myocommata which was soluble in 5% sodium chloride solution was between 21% and 51%, and it is therefore evident that the proportion of this tissue will greatly influence the total soluble protein of fish muscle. The soluble protein in dissected myotomes from cod which varied from 10 to 25 inches in length did not vary significantly (97.1% to 97.8%) in fish at a given time of the year. However, there was a definite seasonal and size trend. This is shown by the findings that, (1) the insoluble protein in the myotome fraction of cod greater than 29 inches in length was greater than that of those less than 29 inches in length, and that there was a seasonal increase in this fraction which was most pronounced about January. It is generally recognized that cod become sexually mature when about 28 inches in length. The very pronounced insoluble protein rise and soluble protein fall which occurs in large mature cod is influenced by shortage of food which occurs about November to February. Since the total protein did not vary from August to November, there was a definite increase in insoluble protein which must have occurred at the expense of the soluble protein. Some of the muscle protein must change its properties and become insoluble in neutral salt solution. This change is also indicated by a visual whiteness or opaqueness and lack of the translucent appearance which is normally apparent at other times of year.

SEASONAL DISTRIBUTION OF COLLAGENS, AND GELATINS DERIVED THEREFROM, IN HERRING

In connection with a problem involving a practical solution to the undesirable "gelling" which occurs in so-called "late-run" herring, MacLeod, Idler and McBride (MacLeod, 1959) studied collagens and gelatins in these fish. It was found that the gelatin content of condensed herring solubles as determined by hydroxyproline varied with season (Table 1). Moreover, the gelling of the solubles closely paralleled the gelatin content.

Table 1. SEASONAL DISTRIBUTION OF GELATIN IN CONDENSED HERRING SOLUBLES

Month	Gelatin
	Percent
August	1.06
September	1.50
November	2.03
January	2.41
March	2.18
April	0.89
May	0.89
June	0.82

Further studies showed that collagen, the natural precursor of the denatured protein gelatin, occurred in three different whole herring protein fractions. These fractions were (1) soluble in 0.2M sodium chloride; (2) soluble in acidic citrate buffer (pH 3.5); (3) insoluble and extractable only by heating residual tissues. The results showed that there are great seasonal variations (Table 2). It will be seen that all gelatin-forming collagen fractions increase markedly with maturity and that the total gelatin in whole herring in

Table 2. SEASONAL VARIATIONS IN THE COLLAGEN CONTENT OF WHOLE HERRING

Month	Collagen Fraction	Gelatin	Gelatin
		(mg./100 gm.)	*Percent of total*
February	Salt soluble	210	23.9
	Acid soluble	274	31.3
	Heat soluble	394	44.9
	Total	878	
June	Salt soluble	151.5	31.5
	Acid soluble	151.0	31.4
	Heat soluble	199.0	41.3
	Total	501.5	

February, when the fish are sexually mature, is almost double that found in June.

A further study was made in order to determine which tissues supported gelatin formation, and results of this investigation are summarized in Table 3. This shows that the gelatin is well distributed throughout the various tissues, but that it (1) occurs in greater concentration in some tissues than others, and (2) increases during maturation more in certain tissues. This specific increase is particularly noticable in the skin and scales, head, and to a lesser extent, in the flesh. There is, however, a noticeable decrease in the visceral content of gelatin with increasing maturity.

Table 3. DISTRIBUTION OF TOTAL GELATIN IN WHOLE HERRING WITH MATURATION

Tissue	June	February
Head	163.0 ± 15.0	278.0 ± 23.0
Tail	31.1 ± 6.8	46.9 ± 2.7
Skin + Scales	110.0 ± 14.3	332.0 ± 27.5
Bone	68.0 ± 9.3	65.5 ± 2.5
Viscera	78.0 ± 13.0	35.9 ± 6.5
Flesh	120.0 ± 12.8	176.0 ± 16.3
Milt or Roe		9.3 ± 0.4
Total	523.4	943.6

The same investigators made an initial study of the total gelatin content of the tissues of migrating salmon. Though further studies are necessary, it would seem that the percentage of gelatin in several tissues of migrating sockeye does not change during migration, but that there seems to be an increase in the total amount of gelatin in a "standard fish" (*vide infra*) in the male head, skin, bones and tail. Further work is necessary in order to determine more exactly the detailed nature of these changes in different tissues.

BIOCHEMICAL CHANGES IN MATURING COHO SALMON HELD IN AQUARIUM TANKS

MacLeod and his collaborators (unpublished) have recently become interested in biochemical changes in maturing salmon held in aquarium tanks; that is, under unnatural environmental conditions. It will only be possible to summarize their findings rather briefly here.

In maturing coho salmon the blood non-protein nitrogen did not appear to show any significant trend, and blood glucose levels were extremely variable and also showed no change which may be associated with maturation. This was also true for serum sodium and potassium ions and total blood protein. The only noticeable change was in the free amino acid content, which showed a marked and sudden drop during only the last month (October) of the experiment.

Several interesting facts emerged from a thorough study of the various tissues of these fish. Thus there was no serious fat depletion in the muscle in the 4 months in which the fish were held without food. There was, however, a slow and steady decrease in skeletal muscle protein in the females, but no appreciable change in the males. In general, from the results so far available, it would appear that the changes in fish held under these conditions are by no means of the same order or as dramatic as are those found in actively migrating sockeye salmon.

CHANGES IN NON-PROTEIN NITROGENOUS CONSTITUENTS OF FISH MUSCLES

Some seasonal changes have been observed in the non-protein nitrogenous constituents of fish muscle. Thus a marked seasonal trend in trimethylamine oxide plus trimethylamine has been observed in North Sea herring. In herring there was a steady decrease from about 60 mg. (as N) per 100 gm. of fish in February to about 20 in July and August in herring and from 25 mg. to 7 or 8 mg. in sprats. There also appeared to be a slight and probably significant increase of muscle trimethylamine oxide with size, and hence with maturity, in cod, haddock and whiting [reviewed by Shewan (1951)].

A significant but quite variable amount of the non-protein nitrogen in fish muscles is in the form of free amino acids. Marked variations in the concentration of these have occasionally been observed and correlated with season or maturity. Taurine falls from 340 to 450 mg. per 100 gm. of Atlantic cod muscle in May to July to 100 to 150 mg. in December to February. On the other hand lysine, which occurs in quite small amounts most of the year (0.5 mg. per 100 gm.) rises sharply to between 12 and 15 mg. during the January-February spawning period (Jones, 1954). A rise in the lysine levels in sturgeon muscle has been observed prior to spawning.

SODIUM AND POTASSIUM IONS

With Pacific spring (king) salmon it has been shown that with small (3 to 5 lb.) fish the muscle Na^+ was consistently higher, and the K^+ lower, in spring and summer than in the fall and winter

months, but with 10-pound fish there was no such variation (MacLeod, Jonas, and McBride, 1958). It was also found that there were significantly higher Na^+, and lower K^+, levels in small than in large fish. The reason for this is not clear. The changes in these ions which have been measured in migrating spring and sockeye salmon are rather inconsistent and are confusing. Thus, with Fraser River spring salmon the Na^+ level dropped to one-half that found in the sea fish during the first 100 miles; and thereafter, except in one group studied, the Na^+ ion content rose to that at the start of migration. With migrating sockeye salmon lowered Na^+ and elevated K^+ occurred while the fish were still at sea; but when they entered fresh water these again became normal until the fish reached the spawning grounds where a marked rise in Na^+ and drop in K^+ occurred. Before results such as these can be accurately evaluated it will undoubtedly be necessary to study the effect of hormones on electrolyte balance in fish and distribution of the various inorganic ions in the organs and in the intra- and extra-cellular water.

Changes Which Adversely Affect Quality

Maturation of fish is usually associated with loss of quality. This may occasion economic loss through enlarged milts and roes, low fat content, poor quality of flesh, or other reasons. Probably certain of the complaints that arise from the fishing industry have their origin in these undesirable maturation changes. Thus it is not uncommon to hear of jellied flounders, soft back cod, and halibut with somewhat white and chalky instead of clear and translucent muscle. It is true that certain of these troubles have been traced to invasion of the flesh with myxosporidia, but in many instances few or no such parasites are to be seen on microscopic observation. The only positive instance of such a condition being traced to biochemical changes associated with maturation is that described by Templeman and Andrews (1956), who studied the American plaice. These workers made a very thorough investigation of this change and traced it to a condition of "protein emaciation or impoverishment." Some of the important findings in this study were: (1) The jellied condition was rarely found in immature fish, and the condition was found sooner in females than in males as maturation proceeded. At maturity about 80% of the fish in certain areas were jellied. (2) A greater percentage of jellied fish was found in areas where water temperatures were -0.06° C. to -0.52 C. than in those where temperatures were 0.78° C. to 1.08° C. All data indicated low water temperatures were accompanied by a large proportion of jellied plaice. (3) Muscle protein fractionation showed that highly significant differences occurred in normal and jellied plaice. Thus the average percentage of total nitrogen was 2.58% in normal and only 1.60% in jellied fish. Though the total protein nitrogen soluble in neutral salt solution (5% NaCl) was only slightly lower in jellied fish (88.5% as compared with 92.8%), the actomyosin nitrogen was only 66.2% of the total N in the jellied fish as compared with 77.2% in normal fish. This difference was largely accounted for by both higher "stroma nitrogen" (8.7% as compared with 6.6%) and non-myosin nitrogen (22.2% as compared with 15.5%). The fat content of the jellied fish was a little higher, 1.02% as compared with 0.77%.

It seems that this condition may also appear in cod and haddock. The authors associated it with a failure of fish living in very cold water to recover the body protein which had been converted to milt or roe during spawning, before the gonads were again developing. The undesirable nature of this condition leads to rejection of fish by the fishing industry due to their poor physical appearance and ready loss of liquids as "drip," especially after fillets from them have been frozen and thawed. There is some indication that this condition occurs in Alaska sablefish, and possibly with Pacific halibut. However, data on halibut are confusing since they indicate that the opaque muscle of "chalky" fish has less water and considerably more fat than normal fish, and that the protein content of the chalky fish is usually somewhat higher (Bailey, 1950, 1951).

Lipids

The seasonal distribution of lipids in fish has been the subject of quite extensive investigations, and it will only be possible to mention a few of these. The references cited give adequate coverage of other literature. Milroy (1906) listed three general annual periods in the life of herring, namely: (1) An active 3- to 4-month feeding period in spring and early summer when the spent fish recover and lay down depot fat; (2) a 6- to 7-month ripening period during which feeding activities gradually decline and the sex organs increase in size at the expense of fat, and (3) a 2-month spawning interval when feeding ceases. In general, it appears that the greatest fat decrease in herring occurs after spawning (John-

stone, 1914), and that at any given season of the year there is more fat in older fish (Bruce, 1924).

One of the most interesting studies was that carried out on herring by Channon and Saby (1932). These investigators refer to research of earlier workers who observed a "rapid rise in the percentage of fat . . . in the muscles during the several months prior to the time when rapid growth of ovaries and testes began. This rise was followed by a steady fall and the lowest percentage (of fat) was reached after the fish had spawned some three months later." They recognized six stages of maturity occurring from June until September when the fish were "spent," and their work was confined to this period. Both saponifiable (fatty acid) and unsaponifiable fractions were studied, and the writers used the concept of a "standard fish" for certain of their calculations, a procedure which has been followed in our own Station's extensive researches on migrating sockeye salmon.

Their results showed that the percentage of fatty acids in muscle rose steadily from June 17 for about 5 weeks, and then fell steadily to about the original level with spent fish. The trend in liver and intestine was rather similar, except that there was a steady rise for only about 3 weeks and then a fall to a very small fraction of the initial value after spawning. This trend was spectacular when expressed as grams of fatty acid per standard fish. Their results also showed a steady rise in the average unsaturation (iodine values) of the gonad fatty acids of both male and female fish from about 137 to 200 (just prior to spawning). No such trend was observed in other tissues, which agrees with later work.

A steady loss in cholesterol was noted in the liver and intestines, a gain in gonad cholesterol, and a constant muscle content. The authors also deduced that there was a net synthesis of cholesterol. This substance accounted for from 24% to 85% of the unsaponifiable material in the various tissues. A net loss of 5.68 gm., or about 85% of the fatty acids occurred in the four tissues studied, presumably to supply energy during the non-feeding period.

Lovern (1938) studied fat variations in Atlantic herring, and Hart *et al* (1940) in Pacific herring. Their results showed a marked seasonal trend which is easily explained by summer feeding, sexual development and eventual spawning. Lovern also observed an increase in unsaturation of the oil (iodine values) with increasing oil content. Normally the oil content of dogfish livers varies from about 67% to 74%, and no seasonal trend has been observed with these viviparous elasmobranchs. However, it has been amply demonstrated that there is a very pronounced increase in the vitamin A potency of the liver oil of dogfish with increasing size. (Pugsley, 1939a; Sanford 1945). This marked increase in vitamin A potency of liver oil with fish size, and therefore with maturity, has also been found in Pacific halibut (Pugsley, 1939b). It has been shown that the actual potency of vitamin A in Atlantic dogfish liver oils increases as follows: immature males < immature females < mature males < mature pregnant females. However, this relationship was to some extent misleading since with greater oil content there was lower vitamin A potency.

Migrating Sockeye Salmon

In the early summer of 1956 Dr. W. A. Clemens visited the Vancouver Station and indicated to Dr. Idler the need for chemical studies to determine energy reserves available to Fraser River sockeye salmon during spawning migration. It was soon agreed that this was an important and interesting problem, and with Dr. Kask's permission work was commenced. It is mainly because of Dr. Idler's energy, ability and curiosity that this work has progressed so rapidly. Also, without the skilled biological knowledge and enthusiasm of Mr. Lloyd Royal, Mr. S. R. Killick, and Dr. W. A. Clemens the work could never have succeeded. As it is, the chemical study which will be reported is by far the most detailed and significant which has ever been made on migrating salmon, or indeed on any maturing fish. It must be emphasized that it will be possible to describe only the highlights of this study. Details are to be found in a Progress Report of the International Pacific Salmon Fisheries Commission (Idler and Clemens, 1959) and in a series of papers which either have appeared, are in press, or will be eventually published (Idler and Tsuyuki, 1958; Idler and Bitners, 1958, 1959; Idler, Ronald and Schmidt, 1959; Duncan and Tarr, 1958; Wood, 1958; Creelman and Tomlinson, 1959).

It has been pointed out that the only precise way of measuring biochemical changes in migrating salmon would be to sample them at different stages in their journey without destroying them (Idler and Tsuyuki, 1958). This is obviously impractical, though it is the technique commonly used in mammalian biochemistry. The obvious solution was to sample sufficient fish at several points on the migrating route to assume that the

pooled results of analyses would represent the average condition of all fish. This is referred to as the concept of a "standard fish." This method works satisfactorily for sockeye salmon, but would obviously be more difficult with fish which are of very different size, e.g., spring salmon. Though there were some very valuable earlier studies concerning chemical changes in migrating salmon (Greene, 1919a, 1919b, 1926; Davidson and Shostrom, 1936; Killick, 1955; Rutter, 1902; Miescher-Rüsch, 1880; Paton, et al., 1858), it has been pointed out that these were either carried out long ago when analytical techniques and tools, and, indeed, biochemical knowledge were not nearly so advanced as they are presently. Also, in these studies insufficient fish were used at different sampling locations to make the results obtained really significant from the point of view of calculating energy expenditures. For these reasons, and because of time limitations, this earlier work will not be discussed, and full emphasis will be given to the findings which have emerged during studies of migrating Fraser River sockeye salmon. Even with these considerations it will be impossible to give more than a general overall survey of the work since so much information has already accumulated. The work has been carried out by a number of scientific staff members and some by summer students.

Salmon do not feed during their spawning migration, and since they have little carbohydrate reserve, draw upon their body lipids and protein. Since there are significant changes in weight, especially with female fish, the actual percentage composition of the whole fish, or a given organ, tells only part of the story. Much more valuable, for obvious reasons, is a record of the absolute amount of a given constituent available to the fish. The concept of the "standard fish" already referred to, has rendered this approach possible. While the practical goal of this work has been to determine whether sockeye reach the river with the same energy reserves each year, and to ascertain the dissipation of these reserves up to the time of spawning, the more fundamental approach has involved a detailed study of many of the important chemical constituents of these fish.

Table 4 shows some of the important physical differences which the fish encounter during the Stuart Lake and Chilko runs. The fish sampled were of a pure race, of uniform length, and were taken in chronological order. Twenty to forty 4-year-old fish were collected at each location and were placed in polyethylene bags in ice for not more than 24 hours before being processed for analyses. Table 4 illustrates some of the important physical differences in the Stuart Lake and Chilko Lake runs.

Table 4. COMPARISON OF THE STUART LAKE AND CHILKO LAKE 1956 SOCKEYE SALMON RUNS

Location	Stuart Lake	Chilko Lake
San Juan to Lummi Island (miles)	135	.....
San Juan to Albion (miles)	215	215
Lummi Island to Hells Gate (miles)	170	.....
Lummi Island to Lillooet (miles)	250	.....
Lummi Island to Fort St. James (miles)	640	
Lummi Island to Forfar Creek (miles)	715	.....
Albion to Chilko Lake (miles)	.....	370
Rate of migration (km./day)	43	37
Entry of river until death (days)	37	44
Time spent in river migration (days)	25	19
Time spent on spawning grounds (days)	12	25
Elevation of spawning grounds (ft.)	2270	3830
Body fat at Albion	♂ 13.6% ♀ 14.1%	♂ 14.1% ♀ 14.8%

ENERGY EXPENDITURES

In calculations of energy expenditures, calorie (Cal.) refers to the large calorie or kilogram calorie. One gram of fat has been taken to equal 9.3 Cal. and 1 gram of protein 4.1 Cal. In the time available it will only be possible to discuss a few of the most important features regarding energy expenditures of the 1956 Stuart Lake and Chilko sockeye salmon runs. It is anticipated that the further work which is proceeding on the 1957 and other runs will eventually be published.

In the first case the energy expenditure naturally varies with different parts of the river. Thus it is greater in the interval between Hells Gate and Lillooet than it is between Lummi Island and Hells Gate. Thus it was found that standard female fish used 2.73 and 2.84, and male fish 2.80 and 5.46 Cal./km., over these two different intervals of migration. These differences are undoubtedly due to differences in elevation and in water temperature. Calculations of data obtained between Lummi Island and Fort St. James show that females consumed 1.16 Cal./km./kg. and the males consumed 1.01 Cal./km./kg. This result indicated a body energy expenditure per unit of live weight of fish of approximately 12% more for females than for males in the 1956 Stuart Lake run. This information agrees nicely if it is

assumed that the male and female fish use the same energy per unit of live weight to maintain life during the migration and that the greater energy utilized by the female is mainly to build up the large comparatively high fat content ovaries. The energy expenditure of 58.7 Cal. per day for the female and 22.7 Cal. per day for the male from the time of spawning until death are in agreement with the observed greater activity on the part of the female fish after completion of the spawning act. To summarize, it is evident that the body of the standard female and standard male fish averaged approximately the same energy expenditure including gonad development during migration to the spawning grounds. However, from Fort St. James until death the standard female expended more body energy than the standard male per unit of live weight, and in addition underwent further gonad development. Table 5 shows similar data for the 1957 Stuart Lake run.

Table 5. ENERGY CONSUMED FROM THE BODY TO MAINTAIN 1 KG. OF LIVE FISH DURING MIGRATION TO STUART LAKE

Location	Cal./kg./km.	% more for female
Lummi Island to Lillooet.....	♂ 1.28	♂ 23
	♀ 1.57	.....
Lillooet to Forfar Creek	♂ 0.60	♂ 42
	♀ 0.85	.....
Lummi Island to Forfar Creek	♂ 0.84	♂ 32
	♀ 1.10	.....

The standard female lost 34% of its body weight and 80% of its total fat and protein, and the male lost only 14% of its weight and consumed only 56% of its total fat plus protein from Lummi Island until the time of death. From the energy point of view this represents a depletion of 79% of the Cal. reserves on the part of the standard female and 68% on the part of the standard male.

A brief comparison of the Chilko Lake and Stuart Lake 1956 runs will now be made. The average sizes of the fish of both runs were almost identical when the fish entered the river. The body and fat reserves of the Chilko Lake fish were somewhat greater than those of the Stuart Lake run. The fat reserves of the females were greater than those of the males for both these runs. At Albion the ovaries of the female fish going to Chilko Lake represented 4.01% of the live weight of the fish, while they represented 4.02% of the live weight of the fish in the case of the Stuart Lake run. This indicates the reliability of using the ovary weights to evaluate sexual maturity on the various runs. These values had increased to 7.04% and 7.44% respectively after 11 days migration. The average loss of water during the first 250-mile migration of both 1956 Chilko and Stuart Lake runs was about 10% on the basis of eviscerated fish. Subsequently the males of both races showed large increases in body water; 17.5% of the reserves at Lummi Island for the Stuart Lake migrants and 28.4% of the Albion reserves for Chilko Lake migrants. Females of both runs showed very little change. After spawning until death loss of water occurs and this loss was greater for the Chilko Lake fish of both sexes, males losing 14% of the reserves as compared with 11% for the females.

It has long been known that migrating salmon rapidly exhaust their reserve fat and also, to a lesser extent, their protein. The Stuart Lake females use 95% of their fat between Albion and the time of death on the spawning grounds, while the males use 91%. Corresponding figures for the Chilko fish were 91% and 78%. Stuart Lake females entered the river with more fat than the male fish per unit of body weight and this was sufficient to balance any deficit, but on the other hand this did not occur with the Chilko Lake fish. Stuart Lake females expended 55% of their protein reserves and the males 34% up to the time of death, while corresponding figures for the Chilko run fish were 61% and 42%. This indicates that both sexes of the Stuart run used their fat more efficiently, while those of the Chilko run drew more heavily on their protein reserve. The total fat plus protein consumed was 79% for female fish of both runs, and 69% and 64% for Stuart and Chilko males respectively.

Calculations showed that from Albion until death on the spawning grounds in Stuart Lake 1,396 Cal./kg. for males, and 1,644 Cal./kg. for females, were required to maintain 1 kg. of live fish. With the Chilko run, from Albion to Chilko Lake, corresponding figures were 1,270 and 1,891. Standard females therefore expend 17.8% more energy than males on the Stuart run and 48.5% more on the Chilko run. The visceral fat reserves of the female dropped 79% as compared with 57% for the male between Albion to the spawning grounds on the Chilko run. However, since the entire fat supply of the viscera accounts for only between 1.85% and 2.78% of the total fat energy

expended by the body, the amount contributed by the viscera is not large.

Available data indicate that sockeye salmon may arrive in about the same state of sexual maturity each year. Thus at Lummi Island in 1956 the ovaries represented 3.59% of the live weight of the female and 3.37% in 1957. There is some indication that Chilko Lake males appear to be more sexually mature than Stuart Lake males on arrival at the Fraser River and this may explain why the Chilko males die before they consume as large a proportion of their energy as do the males of the Stuart run.

Some interesting comparisons have been made between data for the Fraser River sockeye salmon runs and those obtained for Amur River keta salmon (Pentegov, Mentor, and Kuranev, 1928). Only comparative data for the Amur River and Stuart Lake runs will be considered here since the conditions experienced during migration are fairly similar (Table 6). Stuart Lake sockeye migrated 43 km./day for 31 days at water temperatures of 60° to 62°C., while the Amur River keta migrated at 44 km./day for the same time at water temperatures of 50° to 55°C. In general, the comparative energy derived from fat and from protein is rather similar for the sockeye and keta salmon runs, with exception of the lower consumption of energy constituents by the sockeye males. The female keta salmon appear to arrive at the river mouth in a greater state of maturity than the female sockeye salmon, but the final ovary development appears to be closely similar. Sockeye salmon, which have greater fat reserves initially, retain more fat than the keta following spawning.

The preceding data have only contained brief highlights of this interesting study of the various races of Fraser River sockeye salmon. These studies are continuing and there is no doubt that a comprehensive report will be available in the future.

Table 6. COMPARISON OF AMUR RIVER KETA SALMON AND STUART LAKE (1956) RUN SOCKEYE SALMON

	Amur River Keta	Stuart Lake Sockeye
	Percent	*Percent*
Protein utilized	♂ 57	♂ 32
	♀ 58	♀ 53
Fat utilized	♂ 99	♂ 91
	♀ 97	♀ 95
Total energy expenditure	♂ 78	♂ 68
	♀ 80	♀ 79
River mouth to spawning grounds	1152 *Km.*	1192 *Km.*
	Percent body weight	*Percent body weight*
Ovaries		
River mouth	7.02	3.59 (1956)*
		3.37 (1957)*
Spawning grounds	17.40	15.70 (1956)*
		13.70 (1957)*
	Percent	*Percent*
Composition at river mouth		
Water	♂ 68.7	♂ 63.7
	♀ 67.0	♀ 63.1
Protein	♂ 21.08	♂ 19.5
	♀ 20.68	♀ 19.9
Fat	♂ 9.19	♂ 13.6
	♀ 11.28	♀ 14.1
	Percent	*Percent*
Composition after spawning		
Water	♂ 85.4	♂ 81.1
	♀ 77.9	♀ 80.8
Protein	♂ 13.66	♂ 15.5
	♀ 15.85	♀ 16.0
Fat	♂ 0.11	♂ 1.9
	♀ 1.57	♀ 1.4

* Data for both 1956 and 1957 runs.

VANCOUVER STATION PROGRAM OF FUNDAMENTAL RESEARCH ON MIGRATING SOCKEYE SALMON

In view of our Station's interest in the whole problem of the biochemistry of migrating salmon, the International Pacific Salmon Fisheries Commission kindly provided a special series of very carefully handled sockeye salmon from the 1957 run. All fish were caught in reef nets to minimize struggle; they were anesthetized in ice water before being bled by cutting off the tails to sever the caudal artery. Heparin was added to the blood which was centrifuged immediately and the plasma transported to the laboratory in dry ice. Individual fish were placed in polyethylene bags

Table 7. SAMPLING DATA FOR 1957 STUART LAKE RUN

Sex	Group	Number of fish per group		
		Lummi Island	Lillooet	Forfar Creek
Male	♂ 1	7	10	10
	♂ 2	6	10	5
	♂ 3	5	10	6
	♂ 4	5	10	4
Female	♀ 1	8	10	14
	♀ 2	7	10	14
	♀ 3	9	10	13
	♀ 4	9	10	14

Table 8. LENGTH AND WEIGHT CHANGES OF SOCKEYE SALMON DURING SPAWNING MIGRATION

Sex		Total fish	Total length	Snout length	Eviscerated fish	Head, skins, bones + tail	Flesh	Sp. gravity
		g.m.	*c.m.*	*c.m.*	*g.m.*	*g.m.*	*g.m.*	
Male	Lummi Is.	2565 ± 42	59.3	3.4	2322 ± 40	881 ± 33	1467 + 21	1.033
	Lillooet	2371 ± 26	59.3	3.9	2185 ± 25	871 ± 10	1302 ± 51	1.024
	Forfar Cr.	2583 ± 51	61.6	5.2	2382 ± 47	1057 ± 10	1304 ± 40	1.012
Female	Lummi Is.	2376 ± 26	57.4	2.8	2151 ± 19	785 ± 15	1335 ± 13	1.037
	Lillooet	2156 ± 4	57.6	3.1	1908 ± 41	765 ± 20	1135 ± 30	1.034
	Forfar Cr.	2168 ± 10	58.2	3.5	1775 ± 10	834 ± 12	934 ± 10	1.015

Table 9. WEIGHT CHANGES IN THE ORGANS OF SOCKEYE SALMON DURING SPAWNING MIGRATION (grams)

Sex		Heart	Liver	Spleen	Kidney	Alimentary tract	Viscera	Gonads	Tail
Male	Lummi Is.	6.10	31.6	2.90	26.0	90.5	157	56.5	64
	Lillooet	5.88	25.4	1.98	23.3	30.1	86.7	82.0	58
	Forfar Cr.	5.80	37.6	3.60	28.1	16.4	91.5	85.4	82
Female	Lummi Is.	5.05	40.2	2.04	21.6	78.3	147	80.1	59
	Lillooet	4.95	44.6	1.50	19.9	26.2	97.2	138	52
	Forfar Cr.	4.58	40.5	2.17	19.7	12.9	79.2	298	77

and frozen in brine at −19°C. at Lummi Island and were placed immediately on dry ice at more distant points. All subsequent handling of the flesh and organs was done under chilled conditions and all samples were kept frozen until analyzed. Table 7 shows the number of fish taken at each sampling site. The gross length and weight changes of sockeye salmon of this 1957 run during spawning migration are given in Table 8. Some of the changes in the 1956 Stuart Lake run have already been discussed. The data show that with male fish there is an initial loss of weight which is eventually made up at the spawning grounds while with the female fish this initial loss is never made up. These changes correspond to earlier observations already discussed. It will be noticed that there is a spectacular increase in snout length, especially with male fish, and on a percentage basis this increase is 52% for the male and 25% for the female. It is of interest that there is a significant decrease in the specific gravity of the fish, both male and female, during migration due to the increase in proportion of water. There is also a spectacular increase in weight of the head, skin, bones and tail in both male and female fish between Lillooet and Forfar Creek spawning grounds. The weight changes of organs and tissues are considered on the basis of grams per standard fish at the three different locations and for each sex in Table 9. In both male and female fish there appears to be a slight and probably significant decrease in the size of the heart. It is difficult to assess the meaning of the small changes observed in liver, spleen and kidney but it will be noted that the female livers were larger than those of the males in general and that the reverse was the case with kidney and spleen. The wasting of the alimentary tract in both male and female fish is extremely marked, and the total viscera decreased markedly with both male and female fish. The increase in weight of the male milts is extremely marked between Lummi Island and Lillooet and thereafter there is hardly

Table 10. EXPENDITURE OF FLESH FAT AND PROTEIN BY MIGRATING SOCKEYE SALMON OF STANDARD LENGTH

Location	Component expended by standard fish (*mg./mile*)	
	Fat	Protein
Lummi Island to Lillooet	♂ 284 ♀ 304	♂ 220 ♀ 216
Lillooet to Forfar Creek	♂ 52 ♀ 85	♂ 104 ♀ 163
Lummi Island to Forfar Creek	♂ 132 ♀ 161	♂ 144 ♀ 180

Table 11. MOISTURE, FAT, PROTEIN AND CHOLESTEROL IN THE FLESH AND HEAD, SKIN, BONES AND TAIL OF MIGRATING SOCKEYE SALMON: (Grams per Standard Fish)

	Lummi Island		Lillooet		Forfar Creek	
	Flesh	H,S,B,T	Flesh	H,S,B,T	Flesh	H,S,B,T
Moisture	♂ 986 ♀ 894	♂ 530 ♀ 466	♂ 921 ♀ 806	♂ 562 ♀ 496	♂ 1020 ♀ 729	♂ 815 ♀ 636
Fat	♂ 137 ♀ 141	♂ 177 ♀ 170	♂ 66 ♀ 64	♂ 138 ♀ 116	♂ 42 ♀ 25	♂ 50 ♀ 36
Protein	♂ 322 ♀ 294	♂ 143 ♀ 128	♂ 267 ♀ 240	♂ 128 ♀ 126	♂ 219 ♀ 165	♂ 168 ♀ 138
Free Cholesterol	♂ 0.377 ♀ 0.342	♂ 0.537 ♀ 0.502	♂ 0.402 ♀ 0.347	♂ 0.571 ♀ 0.496	♂ 0.406 ♀ 0.324	♂ 0.632 ♀ 0.486
Total Cholesterol	♂ 0.428 ♀ 0.387	♂ 0.627 ♀ 0.551	♂ 0.444 ♀ 0.370	♂ 0.727 ♀ 0.609	♂ 0.479 ♀ 0.335	♂ 0.826 ♀ 0.636

significant change. On the other hand, the female gonads undergo a marked weight increase during the whole period of migration.

Table 10 is of interest in that it shows the expenditure of fat and protein of the flesh of sockeye salmon of standard length. The very different values noted are of roughly similar order with male and female fish and indicate a very marked difference in the utilization of the important energy constituents during different stages of the migration. Thus, fish use almost four times as much fat and about twice as much protein per mile between Lummi Island and Lillooet as they do subsequently between Lillooet and Forfar Creek. These differences of course depend largely on elevations and water temperatures.

Table 11 shows the moisture, fat, protein and cholesterol distribution in the flesh and in the head, skin, bones and tail at the three sampling locations. The initial fall in water content, and eventual very significant rise, in the case of male fish, as has been emphasized previously, is brought out well by both these tissue groups. On the other hand, in female fish the water content of the flesh falls throughout migration, but undergoes rather similar change with initial fall and then rise in the case of the head, skin, bones and tail. In both male and female fish the depletion of fat during migration at all stages is very evident. It will also be observed that the head, skin, bones and tail in all instances account for more fat than the flesh. Though flesh protein is depleted markedly during migration with both sexes, there is some increase which undoubtedly is significant in female fish in the case of the head, skin, bones and tail. On a weight basis there was much more free and

Table 12. PLASMA CHOLESTEROL, Ca^{++}, Mg^{++}, $SO_4^{=}$, AND UREA NITROGEN IN MIGRATING SOCKEYE SALMON (Mg./100 G.)

Component	Lummi Island	Lillooet	Forfar Creek
Free Cholesterol	♂ 261 ± 18 ♀ 215 ± 9	♂ 170 ± 17 ♀ 220 ± 24	♂ 134 ± 15 ♀ 77 ± 6
Total Cholesterol	♂ 570 ± 19 ♀ 572 ± 19	♂ 436 ± 38 ♀ 499 ± 24	♂ 394 ± 25 ♀ 202 ± 20
Calcium	♂ 21.6 ± 0.6 ♀ 27.3 ± 0.4	♂ 19.9 ± 1.3 ♀ 23.8 ± 1.1	♂ 12.5 ± 0.8 ♀ 15.5 ± 0.6
Magnesium	♂ 1.58 ± 0.16 ♀ 0.86 ± 0.06	♂ 3.71 ± 0.25 ♀ 2.88 ± 0.18	♂ 2.15 ± 0.11 ♀ 2.18 ± 0.12
Urea N	♂ 6.0 ± 0.3 ♀ 6.2 ± 0.6	♂ 4.3 ± 0.7 ♀ 3.7 ± 0.5	♂ 4.9 ± 0.2 ♀ 4.2 ± 0.4
Inorganic $SO_4^{=}$ as S	♂ 12.5 ± 0.2 ♀ 13.0 ± 0.7	♂ 5.5 ± 0.3 ♀ 3.9 ± 0.3	♂ 9.6 ± 0.3 ♀ 9.8 ± 0.2

Table 13. NITROGEN CONTENT OF MUSCLES OF MIGRATING SOCKEYE SALMON (Grams per Standard Fish)

Fraction	Males			Females		
	Lummi Island	Forfar Creek	Lillooet	Lummi Island	Lillooet	Forfar Creek
Whole muscle	51.462	42.754	34.951	46.966	38.376	26.717
High ionic + stroma	32.479	25.540	22.038	29.470	22.329	16.149
Low ionic	11.677	12.111	8.354	10.769	10.554	6.347
Non Protein N	6.499	6.097	5.340	5.988	5.225	4.362

total cholesterol in the head, skin, bones and tail than in the flesh at all sampling locations.

Changes in cholesterol, calcium, magnesium, sulphate and urea nitrogen in the blood plasma of migrating Stuart Lake sockeye salmon are recorded in Table 12. There is a spectacular decrease in both free and total cholesterol during migration, and this is most marked with the male fish. The total cholesterol content, 570 mg. per 100 gm., is extremely high compared with that normally found in warm-blooded animals, namely 150 to 190 mg. per 100 gm., but similar high levels have been reported for carp blood. The ratio of free to total cholesterol steadily decreased as the males moved up the river and was 0.46, 0.39 and 0.34 at the various sampling locations. It is apparent that the cholesterol level in the blood serum would be a useful indication of the various stages of spawning migration in these fish. The calcium level which is considerably higher than that found in the blood serum of man definitely decreased in both male and female fish during migration. The magnesium content was somewhat similar to that which has been reported for human serum, and there is a significant increase in the early stages of migration. The significant drop in sulphate levels during the early phase of migration, and later rise is rather analogous to the change which has been reported for human pregnancy serum. The figures for urea nitrogen were somewhat lower than those reported for human serum and higher than those reported for carp serum.

A detailed study was made of the protein and other nitrogenous constituents of these migrating salmon. Only muscle tissue was used in these studies since little is known about proteins of other fish tissues. The nitrogenous constituents were arbitrarily divided into four groups as indicated (Table 13). Unfortunately, in this work the method of sampling by freezing, thawing and refreezing samples caused considerable denaturation of the muscle myosin, the denatured protein being therefore measured with the stroma fraction which represented between about 14% and 20% of the total muscle nitrogen. For fish of this type stroma proteins normally represent about 3%. The changes in high ionic strength extractable fraction, which would undoubtedly be principally actomyosin, and in the stroma fraction cannot be considered significant. There does appear to be a significant increase in the low ionic strength extractable proteins during the early stages of migration with a return to about the initial level at Forfar Creek. There is also a tendency for the non-protein nitrogen to increase as taken on the percentage basis during migration. When the nitrogen content of the muscles is considered on the basis of grams per standard fish (Table 14) there are obvious and very marked changes. Thus, the total

Table 14. NITROGEN PARTITION IN MUSCLE OF MIGRATING SOCKEYE SALMON (Percent total N)

	High ionic strength	Low ionic strength	Stroma	NPN	Recovery
					Percent
Lummi Island ..	♂ 45.39 ± 1.04	♂ 22,69 ± 0.825	♂ 17.96 ± 1.66	♂ 12.83 ± 0.51	♂ 98.43
	♀ 42.6 ± 1.64	♀ 22.60 ± 1.21	♀ 20.55 ± 2.26	♀ 12.61 ± 0.95	♀ 98.36
Lillooet	♂ 44.8 ± 0.345	♂ 28.33 ± 0.44	♂ 14.86 ± 0.785	♂ 14.25 ± 0.25	♂ 102.24
	♀ 42.9 ± 0.965	♀ 27.47 ± 0.44	♀ 15.24 ± 0.328	♀ 13.62 ± 0.32	♀ 99.24
Forfar Creek ..	♂ 49.33 ± 0.84	♂ 23.88 ± 0.28	♂ 15.30 ±	♂ 15.28 ± 0.23	♂ 103.69
	♀ 46.24 ± 1.50	♀ 23.75 ± 0.357	♀ 14.25 ± 0.655	♀ 16.33 ± 0.47	♀ 100.57

nitrogen of the whole muscle decreases extremely markedly as does that of the high ionic strength fraction for both male and female fish. There is no significant change in the low ionic strength extractable fraction between Lummi Island and Lillooet but there was a very definite decrease in both male and female fish at Forfar Creek. A slight decrease in the non-protein nitrogen content of the flesh was observed throughout migration for both male and female fish.

In view of the interest which has recently been taken in the distribution of various nitrogenous compounds in the non-protein nitrogen fraction of fish muscles, this was the subject of special investigation in the case of the migrating sockeye salmon. The following substances were identified: comparatively large amounts of trimethylamine oxide, leucine, valine, alanine, glycine, arginine, serine, glutamic acid, threonine and anserine; moderate amounts of creatine, phenylalanine, tyrosine, β-alanine and histidine, and small amounts of proline and aspartic acid. When considered on the basis of the standard fish, most of these constituents did not change greatly at the different sampling locations (Table 15). However, with histidine there was a very strong decrease to about one-fifth the amount found at Lummi Island (Wood, 1958). This could well be due to a recent observation in California that the occurrence of free histidine in flesh of small salmon is linked with ingestion of this amino acid (Lunton, 1958). There is also quite a marked difference between the amount of α amino nitrogen in male and female fish, with a tendency to decrease during migration.

HORMONES IN MIGRATING SALMON

Hormones belong to the family of organic chemical substances known as steroids. There are not only a very large and diverse number of these compounds but their various biological functions are probably equally diverse. An excellent review of some of these substances has recently appeared (Shoppee, 1958). In general, the estrogens are female hormones and the androgens male hormones; in other words, they are substances which bring about the various changes associated with sexual development of females and males respectively. This is a very broad and general designation and of course there are many fine distinctions. The adrenal cortical hormones are another biologically extremely important class of compounds which is produced by the adrenal cortex, the medulla producing adrenalin. Early work on these hormones was associated with cure of Addison's disease but more recently some 41 compounds have been isolated, of which 8 have high biological activity.

These initial brief statements indicate the high complexity of hormone research. The present

Table 15. THE NON-PROTEIN NITROGENOUS CONSTITUENTS OF SOCKEYE SALMON MUSCLE AT DIFFERENT LOCATIONS ON THE MIGRATORY ROUTE

(mg. nitrogen per 100 gm. wet muscle)

Component	Lummi Island	Lillooet	Forfar Creek
α-Amino-N	♂ 94.8 ± 4.7 ♀ 69.8 ± 3.6	♂ 88.4 ± 2.3 ♀ 65.5 ± 2.1	♂ 94.4 ± 5.5 ♀ 81.6 ± 3.2
Volatile base-N	♂ 10.5 ± 1.2 ♀ 9.3 ± 0.7	♂ 7.2 ± 0.3 ♀ 7.9 ± 1.2	♂ 8.5 ± 1.0 ♀ 11.4 ± 0.6
TMO-N	♂ 5.4 ± 0.3 ♀ 5.4 ± 0.7	♂ 5.4 ± 0.8 ♀ 5.7 ± 0.6	♂ 5.3 ± 0.4 ♀ 6.4 ± 0.2
TM-N	♂ < 0.2 ♀ < 0.2	♂ < 0.2 ♀ < 0.2	♂ < 0.2 ♀ < 0.2
Creatinine-N	♂ 4.9 ± 0.8 ♀ 3.7 ± 0.4	♂ 5.9 ± 0.4 ♀ 3.7 ± 0.3	♂ 5.6 ± 1.2 ♀ 3.1 ± 0.5
Creatine-N	♂ 77.9 ± 8.1 ♀ 95.5 ± 11.8	♂ 91.4 ± 5.4 ♀ 114.4 ± 2.8	♂ 95.1 ± 8.7 ♀ 127.0 ± 3.6
Histidine-N	♂ 10.8 ± 1.3 ♀ 15.1 ± 0.8	♂ 2.1 ± 0.5 ♀ 2.7 ± 0.4	♂ 1.3 ± 0.1 ♀ 3.1 ± 0.1
Anserine-N*	♂ 24.8 ♀ 29.4	♂ 31.2 ♀ 27.5	♂ 30.2 ♀ 36.1

* These values were obtained using combined extracts from the four groups.

brief remarks will be confined to the work of Dr. Idler's group on isolation and characterization of hormones in migrating sockeye salmon and the possible biological function of these in fish will be discussed briefly. The work to which I refer is either in process of publication or has been submitted for publication (Idler, Ronald, and Schmidt, 1959a; Idler, Ronald, and Schmidt, 1959b). The substances which will be discussed are cortisol (17 α-hydroxycorticosterone), cortisone (17 α-hydroxyl-11-dehydrocorticosterone) and 17 α-hydroxyprogesterone (Figure 1). In the initial studies about 1,760 ml. of blood plasma was collected from about 100 Adams River sockeye salmon. This plasma is subjected to laborious solvent extraction and other procedures. In this work the recovery of cortisol was 17 μg. and of cortisone 37 μg. per 100 ml. of plasma, with an overall loss of about 30% or 40% during isolation. No evidence for occurrence of aldosterone was obtained, even when sensitive rat assays were used as criteria. It has been pointed out by Robertson (1957) that at full sexual maturity Pacific salmon show extensive degeneration of the pituitary and marked hyperplasia of the adrenal gland, and this has been postulated to play a dominant role in the degenerative changes and death of the fish after spawning. Cortisol occurs in salmon plasma at about twice the level found in human plasma, and cortisone has not been demonstrated in the latter. Studies at the Vancouver Station have shown by two procedures that there is a greatly increased cortisol concentration in sockeye salmon plasma during migration. Total 17-hydroxycorticosteroid levels determined directly on extracts of the plasma have confirmed the greatly increased steroid levels. It is possible that these high levels are associated with the pituitary degeneration, hyperplasia and assumed increased secretory activity of the adrenal glands of salmon noted above. There is little doubt that a planned closer study of 1959 Adams River sockeye may do much to clarify this situation.

It is only very recently that active research has commenced on the androgen content of sockeye salmon during spawning migration. Using milt samples of the 1957 Stuart Lake run of Fraser River sockeye, Dr. Idler and his associates have carried out a rather laborious extraction procedure. Bioassays with chicks, which were carried out by Professor Biely's department at the University of British Columbia showed a significant increase in androgens (as based on comparative testosterone propionate assays) during the first 250 miles of migration. It has not yet been ascertained whether or not the increase occurring in the 415 miles is significant. The values were 41, 106 and 133 μg as testosterone propionate per kg. at 0, 250, and 715 miles.

The need for designing experiments to artificially produce and maintain different blood hormone levels in salmon is evident since eventual proof of the physiological role of these hormones in salmon, and indeed in other fish, must largely depend on such research. So far, attempts to demonstrate presence of the known electrolyte

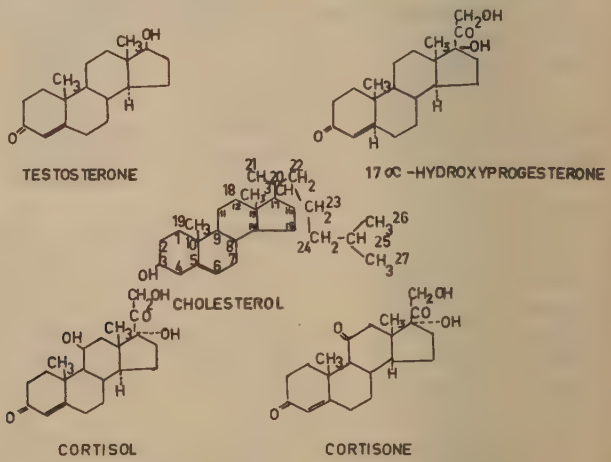

FIGURE 1.

Table 16. THE AMOUNT OF NON-PROTEIN NITROGENOUS CONSTITUENTS IN THE MUSCLE OF THE "STANDARD FISH" (mg. nitrogen per fish)

Component	Lummi Island	Lillooet	Forfar Creek
α-Amino-N	♂ 1391 ♀ 931	♂ 1151 ♀ 743	♂ 1231 ♀ 762
Volatile base-N	♂ 154 ♀ 124	♂ 94 ♀ 90	♂ 111 ♀ 106
TMO-N	♂ 79 ♀ 72	♂ 70 ♀ 65	♂ 69 ♀ 60
Creatinine-N	♂ 72 ♀ 50	♂ 77 ♀ 42	♂ 73 ♀ 29
Creatine-N	♂ 1143 ♀ 1274	♂ 1190 ♀ 1298	♂ 1240 ♀ 1186
Histidine-N	♂ 158 ♀ 202	♂ 27 ♀ 31	♂ 17 ♀ 29
Anserine-N	♂ 364 ♀ 392	♂ 406 ♀ 312	♂ 394 ♀ 337

regulating hormones, aldosterone, corticosterone and 11-deoxycorticosterone, in the salmon plasma have either failed or have indicated that the amounts present are probably insignificant.

In conclusion, it must be emphasized that Dr. Idler and his collaborators are still actively investigating this whole subject.

Discussion

QUESTION: Would you comment on the biochemical function of polyunsaturated fatty acids in marine fishes?

DR. TARR: I don't think too much is known about this. I attended a symposium the other day in Davis concerning fatty acids in fish and their biological function was not discussed. I'm afraid that I can't answer this question.

QUESTION: Economically speaking are post-spawning salmon wasted?

DR. TARR: Yes. I suppose they could be used There is enough protein, and so on, to use as fish meal. I don't know of anyone who would want to eat them. The difficult part, of course, is the collecting of these. They would be highly spoiled in most cases. It would be a very difficult job to collect them. No doubt there are many proteins, and what little trace of fat there is would be quite all right. There is nothing wrong with these fish as far as we know.

QUESTION: Has any work been done on the qualitative changes of gonad protein during maturation, for example, immunological or physico-chemical work? If so, by whom?

DR. TARR: This is a rather big question. There is quite a number of different proteins in these gonads. The male gonads would have protamines on which there was quite a bit of earlier work done. But I don't think most of the work has been done on the mature gonads as far as protamines are concerned. Regarding the types of proteins occurring in the female eggs, I don't know of work done on these—I don't know of any work that has been published. There is a lot of work going on in Japan on some of these subjects. It is possible there has been work done there, but if so, I can't recall it.

QUESTION: Have you any information on seasonal changes in blood and tissue lactic acid of fishes?

DR. TARR: I doubt if this would be of significant importance because it changes more through just simple exertion and through a rise in glycolysis. I would rather doubt whether this would be a very important thing to follow in any case. I don't see why there should be any such thing. I don't know of any information on this.

QUESTION: Please define the terms: standard length and standard fish.

DR. TARR: The standard length was arrived at from the concept that the weight varies as the length of the fish is cubed. I think this is the standard that has been used. Idler has used the idea here that the standard length is the body length minus the snout length. I think that is the standard length. This remains constant during migration. If you look at these results you can see that the

fish can be obtained at a very nice standard length. They don't vary much. I don't know if that is a good answer to this.

QUESTION: Would you speculate on the fate of large quantities of urea in the diet of artificially fed and reared salmon. It has been observed that such fish grow well.

DR. TARR: I didn't know that urea had been fed to fish at all. I know that chickens won't utilize it and I know that ruminants will. I would think and hazard a guess that bacteria might use urea to make bacterial protein and that the bacterial protein would be digested by the fish. I just don't know. This is something that I would like to discuss with the man who is working on it.

QUESTION: Do salmon during migration living at the expense of fat, develop a ketosis?

DR. TARR: I don't think this is known. I don't think so.

QUESTION: Do spent salmon ever recover? Would hormones permit this?

DR. TARR: Well, this of course, is one of the points that is being raised in this whole study. Of course some of the Atlantic salmon do recover, as far as I know. Dr. Clemens might answer this. I don't think that Pacific salmon ever recover. Now I may be wrong there. There may have been isolated cases. But, I don't think they do. Whether hormones would influence this or not, I don't know. It is possible that they would. I don't think there is any information on this. It would be very interesting to try to find out. I would think that withdrawal of hormones might be important but it looks as if a very great excess has more to do with maturation changes.

QUESTION: Have you investigated the changes in glycotytic and/or pentose cycle enzyme activity with migration?

DR. TARR: No, we have done practically no work on muscle or other enzymes in salmon, migrating or otherwise. Most of our enzyme work has been confined to lingcod. I can see that this would be an extremenly interesting problem for anybody interested in energy metabolism in the salmon. It certainly would be very exciting. I don't know anybody who has done anything on this.

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EFFECTS OF PULP AND PAPER MILL WASTES ON THE MARINE ENVIRONMENT

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ABSTRACT

The effect of pulp mill wastes on the marine environment depends on (1) volume and characteristics of the wastes and (2) volume and behaviour of the marine waters receiving the wastes.

Kraft (sulfate) pulp mills recover the salts and organic substances contained in their "black liquor" originating from digestion of wood chips. Sulfite pulp mills generally discharge their waste liquors untreated into an adjacent water course. Other pulp and paper operations, such as debarking, groundwood production and paper making, yield wastes which are chemically quite harmless but contain large amounts of suspended material.

Pulp mill wastes can be harmful to marine life in three ways: (1) direct toxicity, (2) indirect effect through oxygen reduction in the water, and (3) long-term effects on the bottom and water adjacent to it. Kraft mill wastes contain more toxic constituents than wastes from either sulfite or groundwood. Because sulfite waste liquor contains a high percentage of organic constituents, particularly wood sugars, it severaly reduces the dissolved oxygen content of the receiving waters. Most pollution from pulp mills is caused by this effect. Long-term damage to the marine environment is caused by certain cummulative factors which render a gradual change in the aquatic balance of nature.

Three major types of marine systems receive wastes from pulp mills on the British Columbia coast: (1) Inlet, (2) Restricted Embayment, and (3) Tide-Swept Channel. Of nine pulp mills in production on the British Columbia coast, five have been built since World War II and four of these are Kraft producers.

A sulfite pulp mill at Port Alice discharges its wastes into Neroutsos Inlet on the northeast end of Vancouver Island. Pollution is intensive at the surface within several miles of the mill sewer outfall. While bottom fish still dwell in the deep water and some salmon migrate through the inlet to and from tributary streams, the surface water is low in dissolved oxygen and supports virtually no life.

Wastes from the Port Alberni Kraft pulp mill enter Alberni Inlet and have been controlled in strict accordance with prediction calculations of dissolved oxygen supply in the inlet waters. This prediction is based on the amount of water discharging from the Somass River at the head of the inlet and the characteristics of the inlet water.

It has been found most efficacious in an inlet to discharge wastes at the surface, so that river water entering the inlet can flush the wastes out rapidly. In a restricted embayment, it has been found preferable to discharge wastes into deep water outside the bay, so that mixing and rapid dispersion can be achieved. Osborn Bay is an example of this type of system, where a Kraft pulp mill at Crofton discharges its wastes through a diffuser into the deep water of Stuart Channel. The effect of the wastes from this mill on oysters in the vicinity of the outfall is being studied.

A pulp mill at Duncan Bay, near Campbell River, on the northeastern side of Vancouver Island, discharges its wastes into tide-swept Discovery Passage. Tidal currents in this relatively narrow channel are swift and the water is turbulent. Very little pulp mill effluent can be detected outside the immediate vicinity of the pulp mill outfall.

INTRODUCTION

British Columbia is dependent on its forest resources for its primary industry. Pulp and paper mills convert a substantial proportion of the cut timber to the finished or semi-finished cellulose products. Because the sea coast offers many advantages in low-cost hydroelectric power, economical water transportation, and proximity to the lush forests of the seaward side of the mountains, all of the existing B. C. pulp mills are located on tidewater (Figure 1). Nine large pulp mills are in operation, four of which have recently undergone major expansions. Five of these mills are located on Vancouver Island, all having deep-sea approaches.

British Columbia pulp mills are located on one of three general types of marine features, - (1) inlet, (2) restricted embayment, and (3) tide-swept channel. All these marine areas have the general estuarine characteristics where fresh water added from precipitation and runoff exceeds that lost to evaporation. As a result, there is always a shallow brackish layer on the surface of a much deeper and denser sea water layer. This is a property of much importance in the behaviour of wastes added to these waters.

Figure 1. Chart of the British Columbia Coast showing the location of pulp mills.
Note: Production at the Woodfibre pulp mill has been suspended indefinitely.
All expansion programs shown were completed by the end of 1958.

Industrial pollution of marine waters is of considerable concern to the economy of British Columbia. About 10 percent of its resources come from the sea. Among these resources, the Pacific salmon fisheries are the most important. Five species of the Pacific salmon, genus Oncorhynchus, spawn and spend the early stages of their life history in fresh water. Then they migrate to sea by way of the many streams. Pollution can create a serious hazard to these downstream migrants and to the adults returning after anywhere from 1 to 5 years at sea, depending on the species. Because pulp mills are often located near the head of an inlet into which a fairly large-sized stream supporting runs of salmon and trout, may be flowing, the pollution barrier from the mill wastes may be located where it can be most devastating. In addition to these anadromous fishes, which are most vulnerable to industrial pollution, there is a hazard also to the various pelagic fishes, such as Pacific herring, smelt, and capelin. These come inshore to spawn and may enter the lethal zone created by pollution. Molluscan shellfish, particularly oysters in British Columbia, because of their sedentary nature and their shallow-water habitat, can be seriously affected by industrial pollution.

Mr. A. D. Mitchell of MacMillan and Bloedel, Ltd., Port Alberni, made the weekly determinations of DO and BOD on the waters of Alberni Harbor and BOD of the Kraft mill effluent. These data and other information provided by the pulp mill companies are gratefully acknowledged.

PHYSICAL CHARACTERISTICS OF THE MARINE ENVIRONMENT

The sea differs basically from fresh-water lakes in its vastness, high salt content, and effect of tides. In estuarine areas, the fact that sea water is quite dense compared to river water has an important bearing on the structure of these waters. Fresh water flowing over the surface of sea water with a relatively high concentration of salt does not completely mix but forms a stable brackish layer. This comparatively fresh water is separated from the deeper sea water by a layer of rapid density change, known as the pycnocline. Because water increases in density with increasing salt content and decreasing temperature, the pycnocline is intensified by a large temperature decrease with depth normally present in summer.

Runoff as it enters the sea flows along the surface of the sea water entraining sea salt at its lower boundary during its seaward movement. This salt is then mixed upward in the brackish surface layer by turbulence. There is virtually no mixing of fresh water downward through the pycnocline, unless intensive mixing by wind or tidal turbulence occurs. To replace the salt removed from the deep seawater layer by entrainment, there must be a net movement of sea water up an estuary below the pycnocline. Theoretically, this must occur in order to maintain a salt balance in the estuarine system so that all the sea water is not ultimately replaced by fresh water. Current measurements in such "two-layered" systems as well as model observations have shown this deep-water, landward flow to actually occur.

The foregoing physical characteristics of an estuary are of considerable importance in waste disposal problems. They point out several factors: (1) Vertical mixing of wastes released at the surface can only occur to any significant degree in the upper layer above the pycnocline. (2) Horizontal mixing in the upper layer can be widespread. (3) Wastes discharged at the surface will experience a continuous seaward flow as a result of the "flushing effect" of the fresh water outflow in the surface layer. (4) Discharge of wastes in the deep landward-flowing seawater layer can lead to intensive pollution in deep water at the head of an estuary. Such a condition may result in pollution near the surface during later years, especially if the deep water is mixed toward the surface by a dredge or some other disturbance.

Besides runoff, the effects of tides and winds are important in the dilution and dispersion of wastes from coastal marine waters of British Columbia. The relative magnitude of these effects varies in the three different marine systems discussed in examples given in subsequent sections.

CHARACTERISTICS OF PULP AND PAPER MILL WASTES

Wastes from the pulp and paper industry can be categorized into three basic groups: (1) Spent sulfite liquor (SSL), (2) Kraft (sulfate) mill effluent (KME) and (3) Groundwood wastes. All the wastes consist basically of fresh water to which have been added various proportions of organic constituents from the wood being pulped, salts used in the digestion process, and fibers which escape into the effluent.

In the sulfite pulping process, a bisulfite cooking solution is added to wood chips, which are digested with heat under pressure for several hours. Depending on the type of base used in the cooking liquor, - calcium, magnesium, or ammonia, - the spent sulfite liquor may be recovered for its salts and organic content or it may be wasted into an adjacent water course. The calcium bisulfite cooking liquor is not amenable to recovery because of various technical problems involved. A magnesium bisulfite cooking process has been developed to a point where recovery is both economical from the pulp mill operation viewpoint and desirable from the pollution prevention viewpoint. Recovery from the ammonium bisulfite process is technically feasible, but it is not economical and is only carried out as a pollution abatement measure.

Spent sulfite liquor consists of a large number of organic constituents, among which the major ones are lignin (about 50%) and hexose and pentose sugars (about 15%). The lignin is relatively inactive and breaks down only slowly in natural waters. However, the sugars contribute to the high BOD (biochemical oxygen demand) of SSL. There are no highly toxic constituents in SSL.

The Kraft process of pulp manufacture is also a chemical technique, where the wood chips are digested at elevated temperature and pressure with a solution containing sodium hydroxide, sodium sulfide, and sodium sulfate as the active ingredients. Following digestion, the "black liquor" is removed from the fibers by a screening process and is then put through a series of evaporators for recovery. The organic constituents are burned in incinerators with a substantial recovery of heat to the plant. The salt content of the black liquor is recovered and recycled for future use. Only those wash waters from the screens, which are too weak for recovery, are passed into the sewer and ultimately into receiving waters. Generally wash waters are also recycled through the screening and washing units. Kraft pulping wastes may exert a toxic and/or oxygen depressing influence on receiving water courses. Combined wastes from a Kraft mill may contain such highly toxic constituents as hydrogen sulfide, mercaptans, resin acids and soaps. Oxygen consuming organic constituents may also be present, but in much smaller concentration than in spent sulfite liquor. It is generally estimated that there is a ten-fold difference between BOD of Kraft mill effluent and that of spent sulfite liquor.

Groundwood pulp contributes relatively little in the way of chemical pollution of receiving waters. Because groundwood pulp is formed by a purely mechanical process where the wood is reduced to a pulp-like mass by forcing the log against a revolving stone, the main contribution of groundwood pulp effluent to pollution is in the fibers that escape to the sewer. Some soluble materials from the wood are added to the sewers, but these organic constituents contribute to a relatively small BOD.

Most pulp mills carry out some form of bleaching for brightening their pulp. Bleaching operations involve various stages with the use of sodium hydroxide, sodium hypochlorite, and hypochlorous acid. Other reagents such as hydrogen peroxide and zinc hydrosulfite may be used for specialized bleaching purposes. The effect of bleaching reagents on receiving waters is comparatively small. Usually acidic with some oxidative power, the bleach effluents can partially neutralize the alkaline wastes from the digestor section of the pulp mill.

Other wastes, which are mixed in with the foregoing major effluents, include barker wastes, paper machine "white water", evaporator condensates from the Kraft process and general mill sewage. Barker effluents can be rendered virtually harmless by efficient screening. White water from paper machines contains a large amount of fine fibers in suspension which imparts to the effluent its milky appearance. Except by the use of "save-alls", which do not constitute an economic gain for the company, the very fine fibers are difficult to recover.

HOW THE MARINE ENVIRONMENT IS AFFECTED BY PULP MILL WASTES

There are two types of effects of pulp mill wastes in terms of time when they become apparent in the marine environment: A. Immediate and B. Longterm. The immediate effects are those which are manifested in physical and chemical changes of the water immediately after receiving wastes without the later consequences to the bottom, water and biota. A full measure of the immediate effects can be obtained as soon as the inflow of mill wastes and the water system have reached an equilibrium. At that time, there is an equal amount of effluent

being flushed out of the receiving waters to that entering. This balance shifts seasonally as the factors, such as runoff and winds, determining the flushing characteristics of the marine system change.

The long-term effects are those which occur gradually as a result of changes in the ecological conditions incurred by accumulations of certain undesirable materials. This is especially true of the effect of deposition of particulate materials from mill wastes on the bottom. Fibers and other solid mill wastes blanket the bottoms of marine waters and render them unsuitable for development and growth of marine benthic organisms. Populations of bottom-dwelling animals gradually shift from the sensitive species to more tolerant forms, and as conditions deteriorate further, even these forms may not be able to survive.

A. Immediate Effects

1. Chemical changes - inasmuch as most of the waste from pulp mills is chemical in nature, there are important effects which produce chemical alterations in the receiving waters.

(a) Dissolved oxygen concentration - The principal effect of pulp mill waste is the reduction of dissolved oxygen concentration in receiving waters. Two types of oxygen demands are imposed on the water by SSL, - (1) "immediate oxygen demand", which is caused by the chemical reactions between oxygen and reduced sulfur compounds, mainly sulfur dioxide; (2) biochemical oxygen demand (BOD) due to the aerobic decomposition of organic constituents in the receiving water. In ordinary 5-day BOD determinations, the IOD amounts to about 11% of the total oxygen demand (Washington State Pollution Control Commission, MS).

(b) Hydrogen-ion concentration - The introduction of highly alkaline Kraft digestor wastes or acidic bleach plant wastes can produce changes in pH of the receiving waters. An indirect effect of pulp mill wastes on the pH of marine waters results from the shift of the bicarbonate - carbonate balance by the liberation of carbon dioxide from the decomposition of the mill wastes. However, sea water has a natural defense against large pH changes because of its high buffering capacity arising out of the presence of weak acid salts, bicarbonates and borates for example.

(c) Increase in other chemical constituents - Large amounts of dissolved organic and inorganic substances, such as lignin sulfonates, calcium salts, and sulfur compounds from SSL; and caustic sodium salts, lignin, mercaptans and resin acids from KME, are added to sea water in pulp mill wastes. This increases the concentration of these constituents in sea water with possible toxic effects on marine organisms.

2. Physical changes - Certain physical properties of the sea water receiving pulp mill wastes are changed as a result of the physical nature of the wastes.

(a) Suspended materials - The suspended load of the wastes may be carried in the receiving waters for some distance but it generally settles out into sludge banks near the outfall. These settled solids contribute mainly to the long-term decay of the system.

(b) Color - The brown effluent contributes to the discoloration of the receiving waters. Secchi disc reading of 25 ft in the clear water of an inlet is reduced to less than 5 ft near an outfall from a sulfite pulp mill. This has a considerable ecological significance in that light penetration is reduced to 1/4 or 1/5 of normal depth, and phytoplankton production suffers accordingly.

(c) Temperature - A temperature rise in receiving waters can result, depending on the volume and temperature of effluent and that of the receiving waters.

Other physical properties undergo changes but are probably less important. For example, surface tension of sea water is lowered by the SSL and much foaming can occur under the action of wind stirring.

B. Long-term effects

The build-up of materials, which settle out from pulp mill wastes, on the bottom of a marine system contributes to a change in bottom ecology. In the inter-tidal zone, the exposed rocks and gravel are often plated over with pitch-like substances from mill wastes making this usually-productive region unsuitable for the settling of invertebrate larvae. Through this adverse effect on the sessile fauna, the food of many fish is eliminated or heavily reduced. Moreover, the reduction of dissolved oxygen by decomposition

of settled organic substances in the bottom material and in the water adjacent to it makes this zone entirely unsuitable for bottom fish.

While a complete change of water in a marine system following cessation of waste input might rapidly restore the original ecological conditions in the water itself, it would probably take a much longer period for the ecological balance between the bottom and the water to be restored. Long-term effects are difficult to assess over short periods. The error is often made in making one or two surveys after a particular polluting source is instituted and assuming that, if conditions are alright then, they will not change thereafter. Monitoring of physical, chemical, and biological conditions should be carried out annually. Changes are very subtle and creep up insidiously.

SULFITE PULP MILL POLLUTION IN NEROUTSOS INLET, B. C.

Neroutsos Inlet is the southeast arm of Quatsino Sound on the northwest side of Vancouver Island (Figure 2). A typically-deep B. C. inlet, it has a relatively small runoff with poor flushing characteristics. At Port Alice, near the head of this inlet, a sulfite pulp mill has been in operation since 1917. It releases its untreated mill wastes at the surface of the inlet, and has created one of the severest cases of marine pollution observed on the B. C. coast. The area was surveyed in August 1957 to study some of the characteristics of sulfite pollution in a marine area and its effect on the fisheries (Waldichuk, 1959).

The salinity and temperature distributions, shown in vertical section in Figure 3, are typical of a B. C. inlet in late summer. The thin layer of brackish water near the surface becomes warmed during the summer giving a large temperature gradient from the surface to 30 ft depth. The combined effect of low salinity and high temperature at the surface produces a large density gradient in the near-surface layer. A large amount of energy is required to mix through this pycnocline, so that under prevailing summer conditions the SSL flows seaward in the surface layer with very little mixing into deep water.

The dissolved oxygen picture, shown in the lower part of Figure 3, is significantly different from that of typical inlet conditions. At the head of the inlet, there is a large vertical gradient in the surface layer of water with less than 2.5 mg/l at the surface increasing to saturation levels at 20 to 30 ft depth. A decrease occurs again in the deep water to less than 2.5 mg/l. The generally low DO in the bottom water is normally present in most B. C. inlets as a result of slow flushing. Typically of normal inlet conditions, there is a high DO at 15 to 20 ft depth in the water at the entrance to Neroutsos Inlet. This is the result of phytoplankton activity releasing oxygen in their process of photosynthesis. The DO maximum at about 25 ft depth at the head of the inlet may also be caused by a thin layer of phytoplankton, but this has not been substantiated by actual plankton sampling.

Hydrogen-ion distribution through the inlet (Figure 4) follows a similar pattern to the DO with a low pH at the surface in water outside the mill, relatively high values at intermediate depths of 50 to 100 ft and a low pH in deep water. Highest pH levels were measured in the shallow, sub-surface layer at the mouth of the inlet where presumably photosynthesis was high and carbon dioxide was removed from the water.

Spent sulfite liquor is confined mainly to a thin layer of about 25 ft depth at the surface (Figure 4). Concentrations, exceeded 600 ppm at the surface in that part of the inlet adjacent to the pulp mill, and decreased to 30 ppm at a distance of 10 nautical miles from the mill. However, at depths greater than 20 ft, SSL concentration was generally less than 20 ppm even in waters near the pulp mill. Concentrations of less than 10 ppm in the deep water are not considered significant, inasmuch as inshore sea water has substances which respond to the nitrosolignin test (Pearl and Benson, 1940). A "background" value of apparent SSL, can be in the range 0 to 10 ppm. Cells of relatively high SSL appear in the upper 50 ft. These are apparently related to the circulation and mixing process in the inlet.

A small oceanographic survey was conducted by Hutchinson and Lucas (MS) in August 1927. Comparison of their data with those collected in 1957 show some remarkable differences. This is especially true of the dissolved oxygen concentrations observed during the two periods (Figure 5). While the deep water showed similar DO concentrations, the surface and near-surface data were quite different. The very low DO

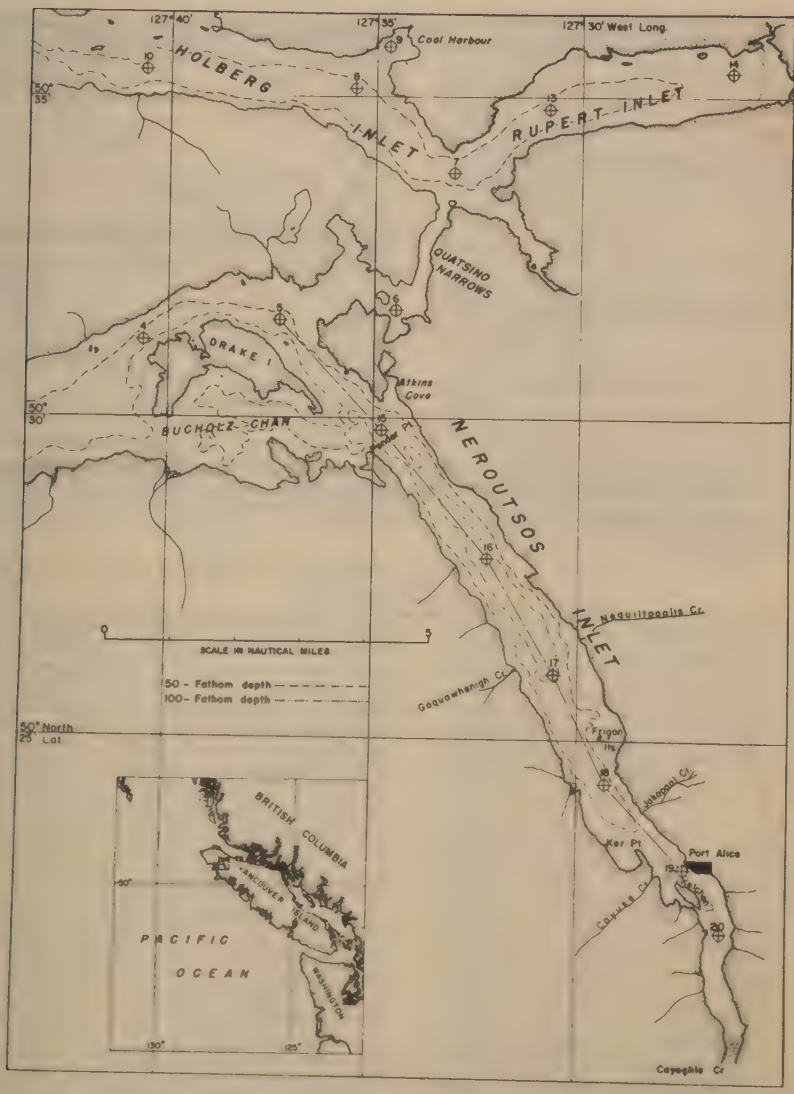

Figure 2. Chart of Neroutsos Inlet in Quatsino Sound with positions of stations occupied 15-19 August 1957.

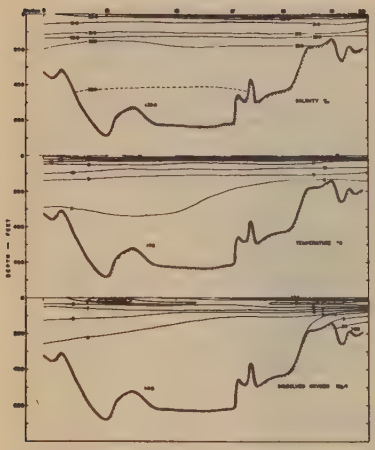

Figure 3. Longitudinal section through Neroutsos Inlet showing the distribution of salinity, temperature and dissolved oxygen.

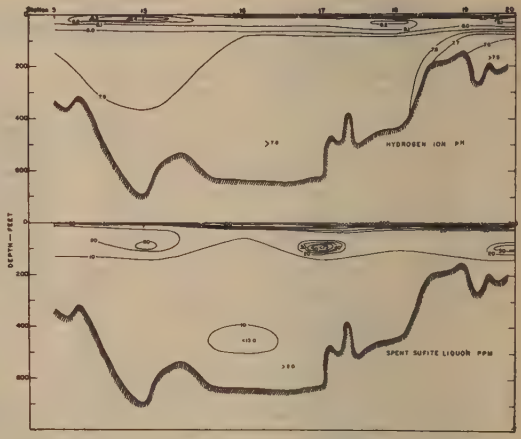

Figure 4. Longitudinal section through Neroutsos Inlet showing the distribution of hydrogen ion concentration and spent sulfite liquor.

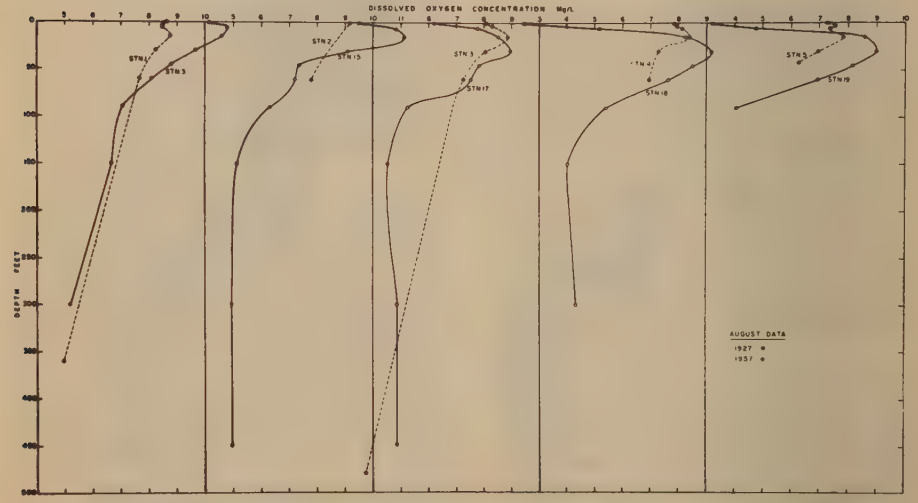

Figure 5. Profiles of dissolved oxygen concentration with depth in Quatsino Sound and Neroutsos Inlet during August 1927 and August 1957.

observed at the head of the inlet in 1957 was absent in 1927. Part of this difference may have been due to lower pulp production during 1927 and dissimilar weather conditions prevailing prior to the two surveys. However, there may have been also a long-term effect on the oxygen balance in the system.

Judging from observations on the aquatic fauna, especially intertidal animals, there was a definite adverse effect on the biota in 1957. Surface water appeared virtually sterile with no fouling organisms on pilings of waterfront structures in Port Alice Harbor. Small fish, commonly observed in the light from the survey vessel during night observations, were absent. In contrast to this, Hutchinson and Lucas concluded in 1927 "(1) That the chemical and physical properties of the water in South East Arm, Quatsino Sound, were well within the limits of sea waters of the continental shelf at the time of this investigation; and (2) that organisms such as are characteristic of similar regions were present there". It would appear that in this inlet, a long-term effect of the 40 years of pulp mill operation had taken place.

KRAFT PULP MILL POLLUTION IN ALBERNI INLET

Alberni Inlet (Figure 6) is another typical B. C. inlet, but it differs from Neroutsos Inlet in that it receives a relatively large runoff from the Somass River at its head. A Kraft pulp mill is located at Port Alberni at the head of the inlet. The oceanography of Alberni Inlet was studied by Tully (1949) as a basis for predicting pollution from a proposed sulfite pulp mill in the inlet. It was shown that the extent of pollution from the mill wastes would depend on the Somass River discharge, which is the chief flushing mechanism. For a relatively small sulfite mill producing 135 tons per day, it was shown that there would need to be a shutdown period of 3-1/2 months every year if intolerable pollution is to be prevented. A possible solution could have been a dam on the Somass River or its tributaries to control the discharge at the required level the year around. The pulp mill company concerned chose to solve this problem by installing a kraft pulp mill instead of a sulfite mill. This mill commenced operation in 1948 and, from then until 1957, it had a modest production of 230 tons per day. During the latter year, expansion was completed to double its production in bleached kraft pulp and commence newsprint production of about 600 tons per day. This overall production was very near to the threshold level of oxygen supply in the receiving waters. Oceanographic surveys have been conducted in Alberni Inlet following the first five years of pulp mill production in order to evaluate the existing conditions and predict the increase in pollution from expanded production (Waldichuk, 1954; 1956 a). Bioassay on the Kraft mill effluent showed that the maximum safe concentration for 1 week's exposure of young Pacific salmon is 4.8% (Alderdice and Brett, 1957). It was calculated that if sufficient water was available to overcome the oxygen reduction by the BOD of the effluent (Greer, Gillespie and Trussell, 1956) there would be sufficient dilution to overcome the toxicity. Tully's (1949) results were put to use in predicting the level of production which could be sustained without serious pollution. During the early stages of expanded production, the output of pulp had to be juggled with various species yielding low BOD being produced during the low discharge period of late summer. Hydroelectric dams are now being installed on tributary streams to the Somass River so that the water flow into Alberni Harbor can be controlled at a desirable level the year around.

The principle of releasing wastes at the surface into the harbor at some distance from the mouth of the Somass River has been adhered to. Construction of a deep-sea wharf and approach trestle facilitated the installation of a flume to carry the mill effluent to a point as near mid-harbor as practical and into the jet stream from the Somass River. Consequently, stagnation of the effluent in corners of the harbor has been reduced to a minimum.

Salinity, temperature and density distributions (Figure 7) in a section through Alberni Inlet are similar to those of Neroutsos Inlet. There is the typical thin surface layer of low salinity, high temperature and low density. In the shallow harbor area, dissolved oxygen (Figure 8) dropped from surface values of 8 to 9 mg/l to levels less than 5 mg/l at a depth greater than 20 ft. Lowest surface DO was found at points adjacent to the outfall where concentrations of approaching 5 mg/l were noted in August 1957. In deep water, concentrations of DO were between 3.0 and 3.5 mg/l. A low-DO pocket at 400 ft depth near the mouth of the inlet is unrelated to pollution but probably results from decomposition of settled plankton and in-

Figure 6. Chart of Alberni Inlet in Barkley Sound showing stations occupied 8-9 September 1954.

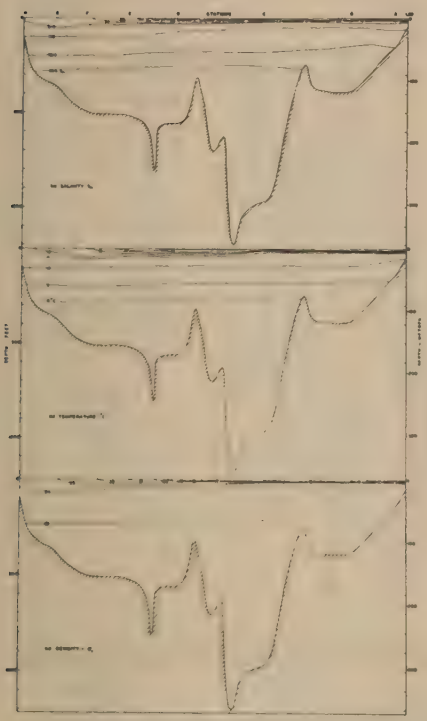

Figure 7. Longitudinal section through Alberni Inlet showing the distribution of salinity, temperature and density (T_t), observed in the September 1954 survey.

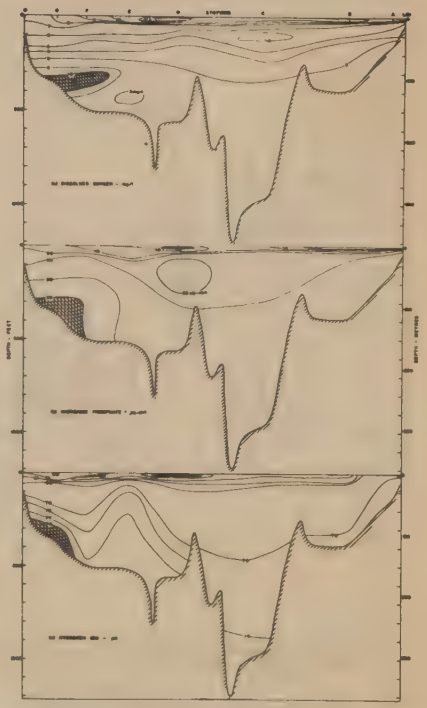

Figure 8. Longitudinal section through Alberni Inlet showing the distribution of dissolved oxygen, inorganic phosphate and hydrogen-ion concentration, observed in the September 1954 survey.

frequent flushing (Waldichuk, 1956[b]).

Seasonal fluctuations in DO at the surface, 10 ft depth and the bottom at mid-harbor are shown in Figure 9. There is a large variation in surface DO from a low of about 5.5 mg/l during August to a high of 13 mg/l in December. Somass River water exhibits DO concentrations only slightly higher than the surface harbor water and undergoes similar week-to-week fluctuations. It was anticipated that the DO of the surface harbor water would be depressed considerably below that of river water during the summer months, but this was not the case, at least for the stations sampled. The region of oxygen sag is probably further down the inlet outside the harbor. This was not observed at stations occupied in the inlet. The wide week-to-week fluctuation of DO observed at 10 ft depth in the harbor is apparently a result of alternation of salt water with fresh water at that depth. During the winter months, the layer of fresh water on the sea surface is quite thick extending, under some circumstances, to a depth greater than 10 ft. This water is saturated with DO at this time. Sea water beneath this layer

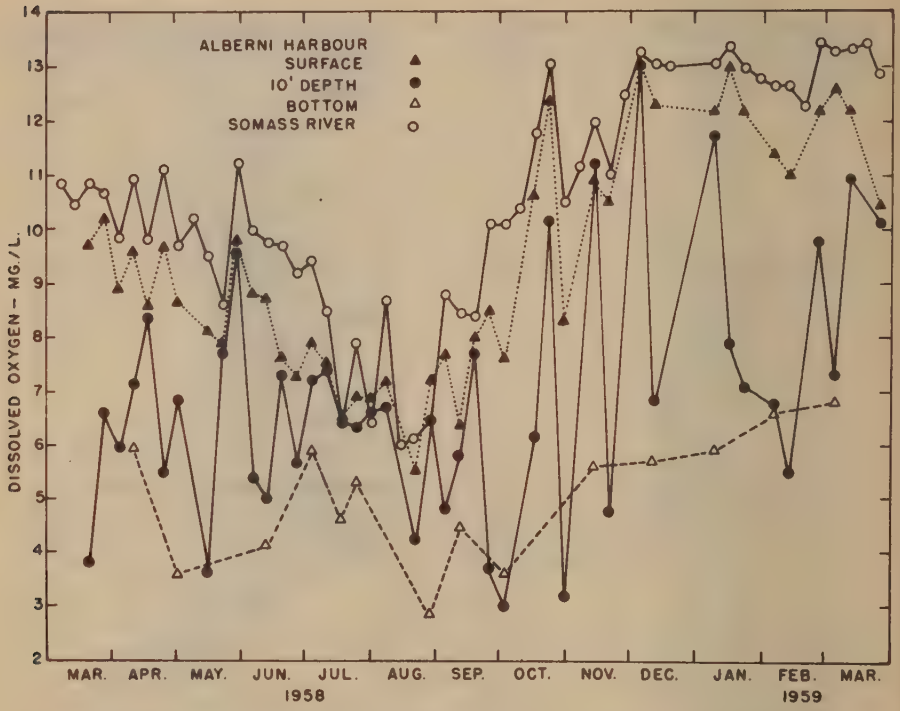

Figure 9. Seasonal variation of dissolved oxygen at various depths in Alberni Harbor (Hohm Island) and in the Somass River, 1958-59.

of fresh and brackish water has a relatively low DO as can be seen from the bottom water concentration at approximately 30 ft depth. Although samples are always taken about 1 hour before daylight high tide, the effect of tide in piling up of fresh water is determined by tidal range which varies considerably from week to week.

Bottom water DO shows smaller fluctuation than that of surface water, but a considerable depression occurs during late summer, with less than 3 mg/l present.

The seasonal picture of BOD of surface water in Alberni Harbor is shown in Figure 10. Some of the large peaks are the result of accidental spillage of black liquor to the sewer or some other such unusual occurrence. Other peaks may be caused by a high organic content of the Somass River water. Weekly BOD data for the mill effluent are shown superimposed on that for harbor water to note any relationship between the two curves. A few peaks of the two sets of BOD data coincide or the high BOD of water follows that of the effluent by about a week. These relationships may be real or fortuitous.

An apparent long-term effect of wastes in Alberni Harbor is becoming evident in the bottom water. Divers have observed an opaque, milky layer on the bottom in the vicinity of the pulp mill. Its appearance was noted mainly in the log storage area of the northeast part of the

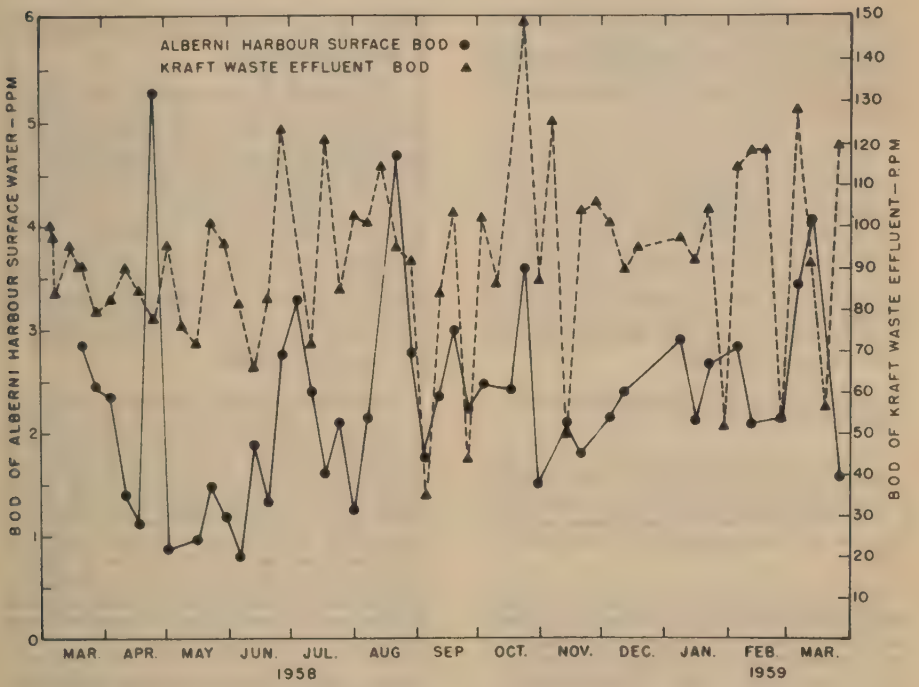

Figure 10. Seasonal variation of BOD of the surface water in Alberni Harbor and of the Kraft waste effluent.

harbor where some stagnation occurs and much solid material settles to the bottom.

Divers have noted bottom fish, described as sea-perch, swimming into the harbor in the clear sea water layer penetrating from the lower part of the inlet at mid-depth. On entering the white layer, the sea-perch apparently suffered no ill effects. However, during low runoff in September, similar fish have been observed to overturn and float to the surface after they were trapped by a large volume of dark brown effluent-containing water moved in by the tide. The turbid layer made log salvage impossible, because of zero visibility, even some weeks after the pulp mill strike of the winter of 1957-58. This suggests that flushing of this deep water does not occur frequently.

As far as the regular commercial and sport fish are concerned, there has been no obvious reduction in salmon and trout runs up the Somass River system since the pulp mill went into operation. During night-time observations, many small fish could be seen milling around in the illumination cast by deck lights. Small flatfish were observed on the tide flats at the north end of the harbor across the Somass River from the pulp mill. A large kill of sticklebacks and shiners occurred during late September of 1956 when a dredge stirred up the bottom along the dock area of the Port Alberni waterfront (Hourston and Herlinveaux, 1957). This was caused apparently by the stirring up

of oxygen-depleted and hydrogen sulfide-saturated bottom materials. A danger of such an event recurring persists because of the build-up of fibers and other solid refuse from the pulp mill and other establishments discharging wastes into Alberni Harbor.

Alberni Inlet is one region where continuous monitoring must be maintained in order to check on the change in conditions. While predictions can be made reasonably well on the fate and effect of the effluent, the other side effects are more elusive and quantitative evaluations on these are difficult to make. The settling of particulate material has a cummulative effect, which may not appear to be serious in the first year or two of operation but can cause serious complications on a long-term basis.

KRAFT PULP MILL POLLUTION VERSUS OYSTERS IN OSBORN BAY

A pulp mill producing bleached kraft was recently constructed at Crofton, Vancouver Island, on the shores of Osborn Bay (Figure 11). It went into operation early in 1958 and is averaging at present a production of about 550 tons per day.

Osborn Bay falls into the category of restricted embayment. Flushing of the bay is relatively slow; but because of its open connection with a larger body of water, exchange is considerably better than that in an inlet. Circulation of surface water within the bay is dominated by winds. However, outside the bay in Stuart Channel, a fairly strong along-channel tidal flow occurs.

A moderate amount of stratification in water structure exists in Osborn Bay. The salinity at the surface is fairly low as a result of dilution from the nearby Chemainus River (Figure 12). During the summer, surface waters become relatively warm. The density difference between surface and deep water prevents any extensive downward mixing. Consequently, dissolved oxygen concentrations of the deep water become relatively low during the course of a summer.

Oyster leases are formed at the approaches to Osborn Bay. For this reason, the surface waters bathing these oyster grounds must be maintained clean. The principle of allowing pulp mill effluent to flow out on the surface of the sea water is not applicable here, inasmuch as the mechanism for good surface flushing is virtually absent. It is necessary to obtain the maximum dilution in the shortest possible time followed by adequate dispersion.

The problem of waste disposal from the pulp mill was studied during its earliest inception. (Waldichuk, 1955). A recommendation was made that the effluent be piped into deep water outside the Shoal Islands and there diffused into Stuart Channel. It was calculated that this channel has a sufficiently large volume of water for adequate dilution if the effluent can be dispersed rapidly. By maintaining the effluent discharge away from the inshore circulation, where tidal current sweeps over the tide flats among the islands and an eddy system prevails in the deep water of Osborn Bay the danger of affecting oysters by mill wastes is reduced.

Observations carried out during winter and summer prior to the pulp mill operation (Waldichuk, 1955, 1958) showed that, under average conditions, the flow was along a northwest-southeast direction in Stuart Channel at all points more than 200 yards seaward of the Shoal Islands. Surveys conducted in the area during 1958 after the pulp mill went into operation confirmed the predicted flow of effluent. Only during periods of easterly and northeasterly winds is there a flow of diluted effluent from the diffuser toward the Shoal Island leases or toward Sherard Pt. on Vancouver Island.

The contrast between effluent distribution resulting from its flow over the sea surface and that occurring when proper diffusion takes place was observed during one period in the early stages of production when the pipeline broke and the discharging end lifted to the surface. Flowing over the sea surface in a sharply defined layer, the effluent spread over the channel in a plume following the direction of prevailing flow of tidal current. The main flow was along the axis of the channel, but comparatively high concentrations still reached the oyster leases on the Shoal Islands. Because mixing of sea water and effluent is an irreversible process, there could be no such stratification of effluent on sea water when the diffuser carried out the initial mixing properly. A reduction of density difference between effluent and sea water allows mixing to proceed rapidly by turbulent diffusion.

Dilution of the effluent in the order of 1:280 was noted in August and 1:550 in November between the diffuser and the nearest oyster leases

Figure 11. Chart of Osborn Bay showing the location of stations occupied 18-19 November 1954.

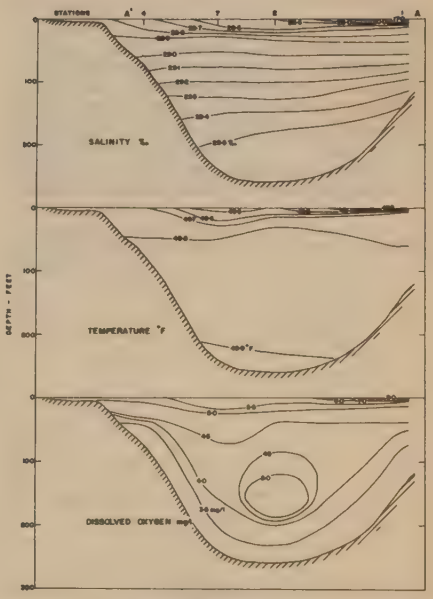

Figure 12. Section through Osborn Bay showing the distribution of salinity, temperature and dissolved oxygen, observed in the November 1954 survey.

1000 yards away. This dilution is 4 to 8 times less than that found to be the minimum required for oysters to be unaffected in research on the York River, Virginia, pollution problem (Galtsoff et al., 1947). The threshold concentration, when the pumping rate of oysters begins to be affected, was estimated to be 1 part of effluent to 2000 parts of sea water in the York River study. However, it is estimated that there has been a 5-fold improvement, and possibly better, in the recovery of kraft wastes during the past two decades.

Dr. D. B. Quayle conducted condition factor studies on oysters in the Crofton area for a number of years prior to pulp mill operation and has recently resumed these studies. Oysters in the area have shown a considerable decline during the winter of 1958-59. Growers believe that this decline has been entirely caused by wastes from the pulp mill. Dr. Quayle's data show that the condition of oysters was low in the whole Stuart Channel region during January and February of 1959. However, his earlier results show that the condition of oysters has been equally low for prolonged periods during previous years. The Pacific oyster (Crassostrea gigas) spawned heavily during the summer of 1958 when sea water temperatures were unusually high. The question has been raised as to whether the oysters fully recovered from the 1958 spawning and whether subsequent feeding conditions were so poor as to prevent the oysters from fattening properly. Field experiments being conducted by Dr. Quayle this spring should provide some information on how oysters are affected by the Kraft Mill effluent at different distances from the outfall, i. e., by different KME concentrations.

The ideal marine location for a pulp mill pollution-wise is the shore of a tide-swept, turbulent channel. An example of such a location in British Columbia is on the shores of Discovery Passage, a narrow channel between northern Vancouver Island and the mainland. A kraft pulp and newsprint mill in Duncan Bay

(Figure 13) discharges its wastes into the swift and turbulent waters of Discovery Passage. As shown in Figure 14, the distribution of salinity, temperature, and dissolved oxygen from top to bottom in this passage is almost homogeneous. The lens of relatively low salinity, high temperature, and high dissolved oxygen in mid channel was probably moved in by tidal currents from a stratified area outside the channel and had not yet been dissipated. Effluent cannot be detected by our available analytical methods outside a radius of a few hundred yards from the outfall.

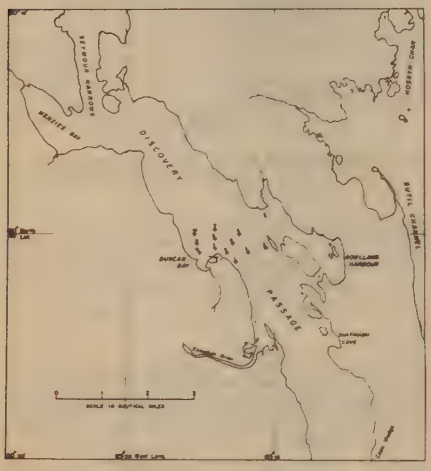

Figure 13. Chart of Discovery Passage showing location of stations occupied 3 - 4 July, 1957.

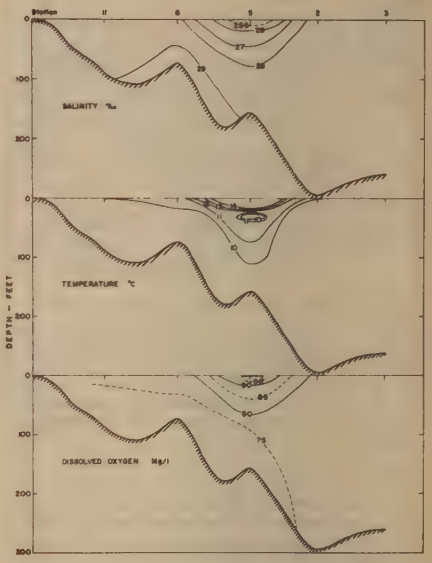

Figure 14. Section across Discovery Passage and through Duncan Bay showing distribution of salinity, temperature and dissolved oxygen, observed during the July 1957 survey.

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THERMAL REQUIREMENTS OF FISH—THREE DECADES OF STUDY, 1940-1970

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ABSTRACT

Three decades of research (1940 to 1970) on temperature relations in fish, and cold-blooded animals generally, are discussed. Speculation on the trends in the next decade are made from a survey of current literature.

Temperature acts in a variety of ways. It may kill, reduce activity, interact with other environmental factors, confine reproduction, and be a directing agent where a gradient exists.

Differences between species in maximum temperature tolerance can be as great as 17°C. (31°F.), and, in overall tolerance, a difference factor of 2.7 times can separate the most tolerant from the least tolerant. Despite ability to withstand low temperatures, slow rates of acclimation appear to result in greater mortalities from cold in nature.

Temperature controls the activities of fish by regulating rates of metabolism (resting and active). The numbing effects of low temperature restricting performance, or the equally restricting effects of high temperature demanding excessive body maintenance are of serious consequence for maintaining continuity of a species. Research on this aspect and the information made available is required if adequate thermal relations are to be prescribed for fish: In order to prescribe thermal requirements, limits to the "safe" reduction of activity imposed by temperature are set forth as a basis for discussion.

Research on the interaction and stress from environmental factors, employing long-term observations, at all stages of development can be expected to reach a new level in the next decade.

INTRODUCTION

Within the general bounds of the last two decades (1940-1959) it has been possible, in retrospect, to see many of the "seeds of thought" for research in temperature relations taking root in the one or two years preceding each decade. Although there is quite a vast literature dating back into the last century which could conceivably bear on the subject of thermal requirements in fish, the research was not tempered in the later knowledge of the considerable effects of temperature acclimation and the consequences of pre-history generally.

Prior to 1940 a great deal of the temperature work was of segmental significance, and difficult to draw into any generalized pattern. Catagorically, it was known that upper and lower lethal levels existed, that acclimatization was a recognized phenomenon, that tolerance differences between species were apparent, and that if differences between internal body temperature and external environmental temperature did exist, these were slight and likely to be no more than transitory (see review by Brett, 1956). Early in the 1940's, and immediately preceding this period, more systematic examination of temperature tolerance relations had commenced, particularly in the school of research headed by Fry (Fry et al., 1942) and in the association of Doudoroff working with Sumner (Sumner and Doudoroff, 1938; Doudoroff, 1942).

Thus, in the decade of the 1940's, although diverse experiments were underway, the emphasis of research in North America was on the extremes of temperature which could be endured and the precise modification of these induced by previous periods of thermal acclimation.

Prior to the close of this decade, Fry (1947) published a broader concept of temperature as an environmental factor, emphasizing the multiple role of temperature and categorizing its action in terms of lethal, controlling (governing metabolic rate), directive (gradient response) and accessory factors. This interpretation provided clarity to the paths of thinking which had been expressed in the literature, and set the scene for approaching thermal requirements through the medium of metabolism and temperature-dependent activities, a pattern of research which has been pursued with intensity in

the 1950's. Indeed, among poikilotherms in general there has been an impressive surge in the study of rate-temperature (RT) phenomena (Bullock, 1955).

Now, with the close of the 1950 decade, and accepting the momentum which tolerance studies and research on temperature-dependent activities undoubtedly carry into the next decade, it is interesting to speculate on the possible trends which will find new accent emerging in the 1960's. Under appropriate subsequent headings, brief summaries of the significance of the past two decades and anticipations in the next are set forth.

Before proceeding with this discussion, some consideration must be given to what is meant by "thermal requirements". The thought behind "requirements" is that of necessity rather than desirability or the maintenance of ideal conditions. It implies setting limits to what must be provided for the organism to permit survival at a level which allows for continuity of the species. Further, at any one time, it must be asked, thermal requirements for what? - for immediate survival, for good growth, for migration, for escape from predation, for successful osmoregulation, for spawning, or what? These are all activities or functions of one sort or another, some required throughout the life cycle, others imposing a requirement at specific times only.

It can be stated that a major requirement for a physical environmental factor like temperature is that it should provide for a level of activity commensurate with maintaining the species at a population level which is more than just a token sample. The particular range of temperature to meet this provision is not fixed, but is dependent on, or at least influenced by, such factors as season, diet, age and size, from egg to mature adult. It must allow for all those activities which relate to maintenance, food procurement and adequate digestion, not at an optimum level, but, to repeat, at a level commensurate with species survival. It is obvious that there can be no simple pronouncement on thermal requirements.

PHASES OF RESEARCH

Temperature-a lethal factor (the 1940 decade)

For any species it is desirable to know just what it can tolerate when faced with the problem of temperature as a lethal factor, acting directly on protoplasmic organization. This outer framework, provided by the zone of tolerance (Figure 1), defines the final boundary between life and death. As recorded in the laboratory, in its upper and lower limits over a complete range of thermal acclimations, the end-points usually used are the median tolerance levels for exposures of time which indicate that mortality from temperature as a primary factor has ceased. It must be recognized that these boundaries do not reveal what can be resisted for shorter intervals, or at the other end of the time-scale, what detrimental long-term effects can accrue from secondary involvements. The lethal temperature than, is a sensitive, useful physiologic end-point when employed in its intended manner.

It is not the intention here to amass the numerous and growing records of tolerance which now exist among a wide variety of species. For this the compilation by Hart (1947), the review by Brett (1956), such texts as Heilbrunn's (1948), and the Handbook of Biological Data edited by

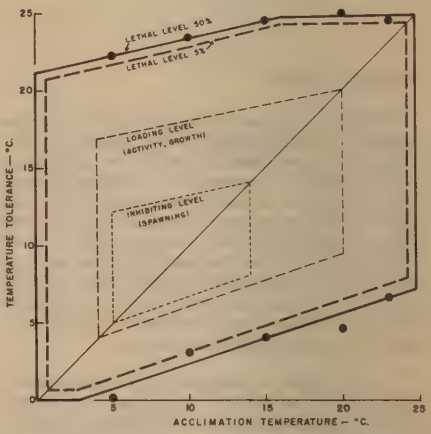

Figure 1. Upper and lower lethal temperatures for young sockeye salmon plotted to show the zone of tolerance. Within this zone two other zones are represented to illustrate (1) an area beyond which growth would be poor to none-at-all, under the influence of the loading effect of metabolic demand, and (2) and area beyond which temperature is likely to inhibit normal reproduction. (From Brett, 1958).

Spector (1956) are useful. A few pertinent points should be made, however.

All species to date, endemic to the United States and Canada, given adequate acclimation, have shown the ability to tolerate temperatures in the region of the freezing point of fresh water.* Rapidly onsetting low temperature, however, can constitute one of the greatest threats to survival when fish are accustomed to relatively warm water because of the inherent slow rate of acclimation to low temperature. There is, therefore, a required time-factor for acclimation which shows marked difference in duration within any given species depending on whether the temperature is rising or falling (Doudoroff, 1942; Brett, 1946).

The ultimate upper lethal levels can differ between species by as much as 17°C (31°F), e.g., Carassius auratus - 41°C (106°F), Oncorhynchus gorbuscha - 24°C (75°F), and the zones of thermal tolerance (as defined by Fry et al., 1942) by a factor of 2.7 times. Such differences demonstrate the very large variation in tolerance that exists, and the quite remarkable extremes which some species can withstand. In recent years, Bailey (1955) has recorded an interesting series of observations on heat-death in a mixed population of fish in a shallow bay in southern Michigan where the temperature rose to 38°C (100.4°F). Such instances of death from upper lethal temperatures are unusual. In nature species are usually found living well within their range of thermal tolerance.

Temperature -- a controlling factor (the 1950 decade)

The penetrating observation made by Fry in 1947 that temperature acts through metabolism on activity opened up a new approach to what was a somewhat dispersed not to say confused field of enquiry. Although there is a lot to be learned by studying the change in rate of any activity at a variety of temperature levels, the understanding and hence interpretation remains obscure without the knowledge of metabolic demand. By demonstrating that the performance or scope for activity was related to the difference between resting metabolism and active metabolism, a means of examining the influence of temperature as a controlling factor was provided. Temperature, then, influences activity by governing the relative rates of energy demand for maintenance, and energy demand for performing essential work (e.g., swimming, digesting, courting, etc.). Since the characteristic feature of a controlling factor is to be operative at all levels, the animal never escapes the assets or liabilities imposed. A striking example of this relation can be taken from recent work on temperature and cruising speeds of young salmon, Figure 2 (Brett et al., 1958). For sockeye the maximum sustained swimming speeds at 10°C (50°F) and 19°C (66°F) are approximately equal but, because of increased metabolic demand at the elevated level, energy reserves would be exhausted 1-1/2 to 2 times as fast at 19°C. This illustrates the common error of thinking that equal levels of performance call forth equal demands on metabolism.

The thermal requirement which can be expressed for any fish in the instance of temperature acting as a controlling factor becomes,

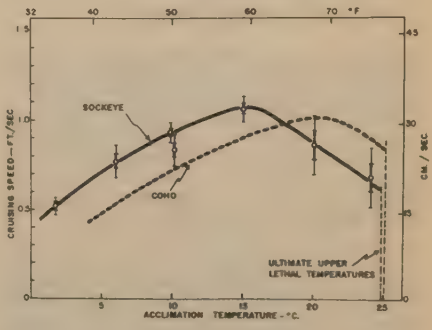

Figure 2. Variation in cruising speed for temperature acclimated underyearling sockeye and coho salmon, illustrating the greater activity of coho near the upper lethal temperature, and the converse relation for lower temperatures. (From Brett, Hollands and Alderdice, 1958).

* The author would appreciate any exceptions to this statement being drawn to his attention. It seems possible that there may be some intolerant endemic species in the most southern regions (but see Fries, 1952).

first, a level which will permit sufficient difference between resting and active metabolism to provide for essential activities, and second, since different species prosper at different levels, it is of distinct advantage to the animal to be at the lower level of metabolic demand for two equal levels of performance since the nutritional demand is reduced. This relation undoubtedly accounts in part for the decline in growth despite increased feeding which may accompany elevated temperatures. Baldwin (1956), while conducting an experiment on food consumption in brook trout (Salvelinus fontinalis) using live minnows, ran into the experimental problem that the trout were comparatively slow at catching the minnows at 17°C (63°F) and virtually incapable at 21°C (70°F), with the result that the trout ran the risk of starvation and energy loss from ineffective attempts at capture in the midst of plenty. This experimental hazard was solved by the simple expedient of clipping the caudal fins off the minnows. It serves to illustrate the relative problem of thermal requirements for the two species, each well within any lethal level.

Temperature -- cellular action, interaction and stress (the 1960 decade)

In the last year (1959) of the present decade, it is intriguing to peruse recent literature for the seeds of new and promising research. A good many lines of endeavour can be seen. Considerable increase in examining responses to temperature change at the cellular level, and seeking the basic mechanisms of acclimation, tolerance, and shifts in metabolic response with temperature can be expected.

For two decades Belehradek's 1935 monograph remained the most comprehensive treatment of temperature and life. This work has now been complimented by two books of comparable stature, that by Precht, Christophersen and Hensel (1955), and the symposium entitled "Influence of Temperature on Biological Systems", edited by Johnson (1957). In Belehradek's time no less than seven theories regarding the cause of heat-death were current. Most of these have been disproven or lost favour, although Heilbrunn (1948) continues to find questionable support for changes in the lipid structure of the cell membrane as the basic phenomenon for thermal adjustment and the ultimate cause of death. Precht et al. (loc. cit.) have brought a refreshing and more inclusive postulation to the fore, based on protoplasmic viscosity, which, despite some possible shortcomings as seen at present (Fry, 1958), holds encouraging promise that a unifying concept for thermal acclimation and tolerance will emerge within the next decade. Their thesis that the thermal stability of the protein molecule relates to the extent of bound water provides a basis for cold-death, through loss of vital water from the cell.

Further, in relation to basic chemical theory, as Johnson (1957) points out, the understanding of the effects of pressure and temperature is emerging in greater significance with the realization "that the physiological effects of increased pressure are subject to profound modification, even reversal, by temperature".

Turning to the relation of the total organism and its environment, the further application of the factor approach (Fry, 1947) to examining the influence of identities like temperature, oxygen and pressure will provide greater analytic insight into the overall mode of action and interaction. There appears to have been some neglect of this penetrating approach in the past, which surely will not be so in the future. If such a relation as, say, the effect of temperature on the resistance of fish to sodium arsenite is under consideration, the difference between the response at 20°C and 25°C might be surprising, simply because temperature had entered the lethal sphere in its own right. A shift in the mode of action would have occurred. If the reverse situation is concerned - the effect of sodium arsenite on the temperature response of fish - then, since temperature is a multiple factor, a variety of questions are actually involved, viz., the effect on thermal resistance, on response to a gradient, on the Q_{10} of some activity, or on the thermal span for regulated osmotic balance. These relate to the effect of temperature when acting as a lethal, a directive, a controlling, or an accessory factor. Temperature can even become its own interacting factor.

With the multiple role of temperature bringing increased attention to the problem of interaction, there will come a natural shift of emphasis away from the consideration of requirements in regard to a particular environmental identity like temperature, but rather to the over-all characteristics of environments which permit high survival of the species. Cocking (1957, 1959) has begun to examine the

significance of long-term temperature effects where accessory factors are undoubtedly involved. Taking a leaf from the excellent research on the lobster Homarus americanus (McLeese, 1956; McLeese and Wilder, 1958), studies on the interaction of temperature, oxygen and salinity can be expected. Work in our laboratory has recently commenced on an analysis of these factors in relation to the resistance of young coho salmon (O. kisutch) to kraft mill effluent. The fruitful pursuit of performance studies is likely to continue, particularly in a comparison of different activities and their relative metabolic demands as influenced by temperature (Brett et al., 1958). Woven amongst these will be the stimulating question of environmental stress, its manner of action and its endocrine involvement (Prosser, 1957; Brett, 1958; Fortune, 1958). The products of this forthcoming decade will provide increased reason to view temperature as a subtle, all-pervading, complex environmental factor.

DISCUSSION

Throughout the research which has been but briefly treated or noted in the preceding comments, it has been possible to see a separating of the parts for laboratory study followed by a return to examining the organism as a whole, inseparably fused in a complex environment. At one time it seemed as though systematic documenting of lethal temperatures might provide a useful basis for establishing limits of distribution in nature. Although grossly apparent, with warm water species tending to have wider or higher tolerance limits, inconsistencies were too numerous for more direct use. By combining this basic information with the further knowledge of the scope for activity which accompanied the extremes of tolerable temperature, more profitable interpretations have been possible. Thus, the two species of Pacific salmon, sockeye (O. nerka) and coho (O. kisutch) have very similar upper lethal temperatures but inhabit quite different regions within the same watershed and exhibit differences in behaviour correlated in part with temperature characteristics of the environment. The possibility of a temperature-dependent causal mechanism became apparent only when it was shown that coho possess an ability to remain active in the region of their upper lethal temperature, whereas sockeye do not (Figure 2). For the lobster, this type of reduced performance is nicely illustrated by a graph relating lethality and inactivity (Figure 3) from McLeese and Wilder (1958). The authors' comment, "Continued existence of the lobster would not be possible in the zones of inactivity although the temperatures themselves are not lethal".

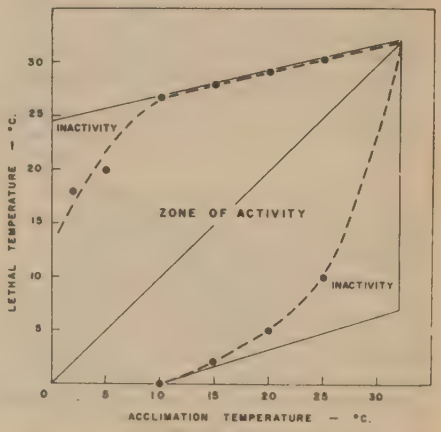

Figure 3. Thermal tolerance diagram for the lobster, showing zones of activity and inactivity where continued existence would not be possible. (From McLeese and Wilder, 1958).

The thermal requirement becomes, for a major portion of life, the need to be in a temperature range within which a fair measure of activity is possible. The phenomenon of temperature compensation in which the metabolic rate is favourably shifted to permit greater activity in poikilotherms inhabiting colder or warmer waters respectively, supports the above thesis concerning thermal requirements. To paraphrase the relation, at prevailing temperatures, the organism cannot afford to be numbed into inactivity nor be heated into the energy-draining maintenance of an excessive resting metabolism.

The homeotherm, by stabilizing internal temperature, despite the frequent cost of increased maintenance, has provided greater scope for activity for a wide range of external

temperatures. The poikilotherm, however, is denied this measure of control, and appears to rely on a fairly wide difference in metabolic rates despite changing temperature, within the range it normally inhabits (Figure 4).

Based on the deductions of the above discussion, and for the purpose of providing some starting point, it is proposed herewith that the upper limit of required temperature for any species of fish should not exceed that which

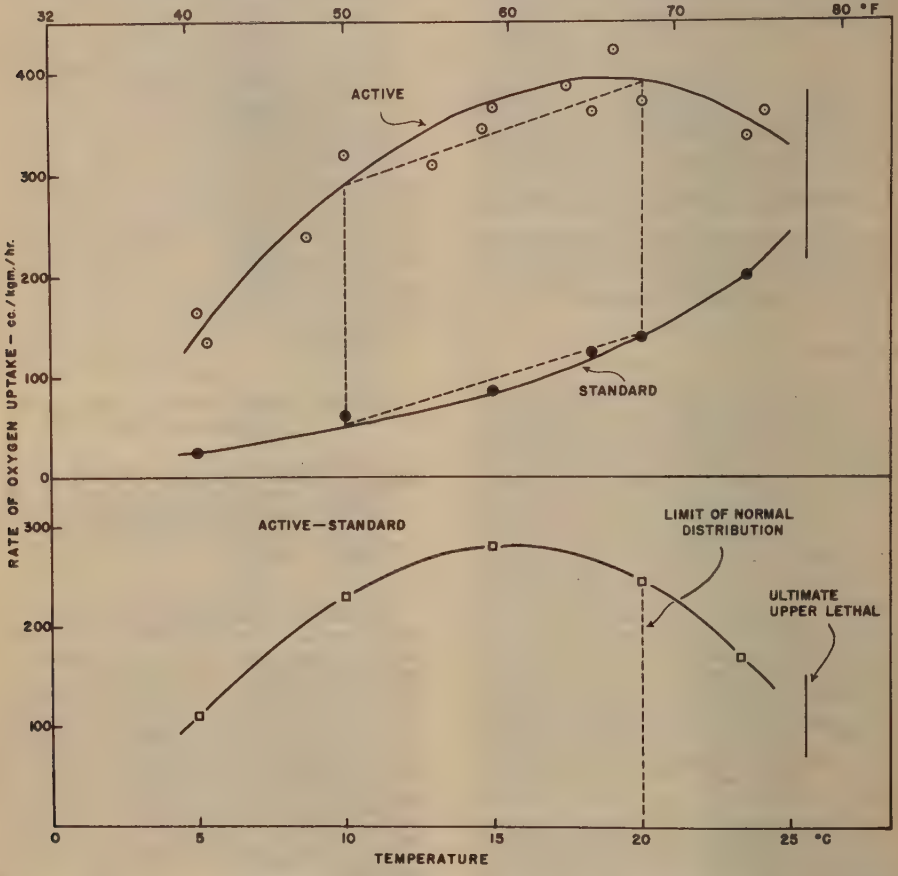

Figure 4. Relation of oxygen uptake to temperature in acclimated speckled trout. The difference between the active and the standard equals the scope for activity (From Graham, 1949). Limit of the normal distribution in warm water occurs at a temperature where the scope is about 4/5's of the optimum. An area in which the difference between active and standard metabolic rates is maintained at a relatively consistent level is indicated, i. e., where the scope is a flattened dome.

would curtail activity below 3/4 of the optimum, i.e., 3/4 of the maximum difference between active and resting metabolism. For those species which can maintain greater fractions of activity into the lethal range, a "freedom" of $3^{o}C$ ($5.5^{o}F$) below the ultimate lethal should be provided. In the case of lower temperature levels, the same metabolic or lethal temperature relation cannot be proposed, since it is annually demonstrated in ice-covered lakes that many species tolerate temperatures of a near lethal order for some months. Such things as infection, disease, parasitism, nutritional demand, and the general problems of competition and predation are often so reduced that a form of "hibernation" is possible near $0^{o}C$.

The above is set forth to invite discussion and appropriate research. It would only apply to the free-living fish, not the embryo, nor the spawning adults, and presumes that other environmental factors remain within normal bounds. Two remaining points should be made. At no time have preferred temperatures been introduced. Such levels can hardly be considered requirements; however, they have received significance in some instances by being related to the temperatures which provide for maximum scope for activity (Fry, 1947). Finally, temperature tolerance studies for every stage in the life cycle are sorely needed (Figure 5), particularly at the level of the developing embryo. Seymour (1956) has recently contributed to this need by a study of the effects of temperature on incubation and hatching in chinook salmon eggs. If the profitable studies on embryonic temperature adaptations in the frog (Rana pipiens) by Moore (1949) and Volpe (1957) can be taken as an indication of the value for similar studies among fish, the concepts of thermal requirements will be considerably abetted.

In conclusion, life is only possible for very brief periods outside the zone of thermal tolerance. Within it, the energy demands of a competitive life, the particular requirements for spermatogenesis or oogenesis, and the need for a relatively stable cellular environment, impose limitations on thermal experience if the species is to survive at a respectable level. Until these subtleties are better understood, provision for a definite measure of activity must be afforded to meet the problem of thermal requirements.

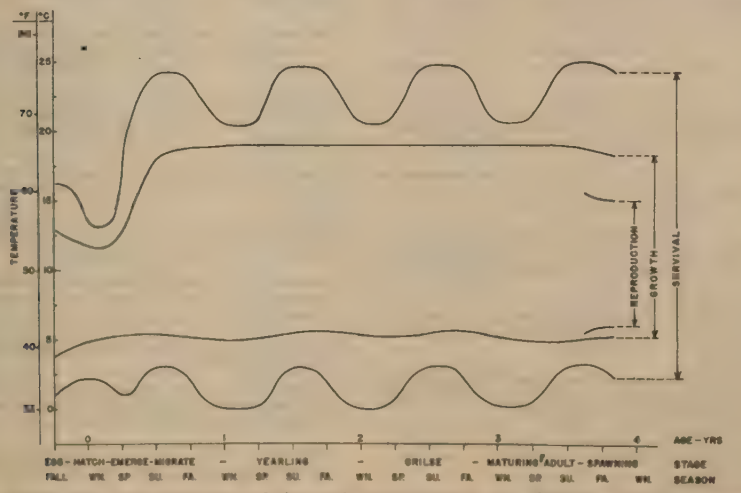

Figure 5. Schematic representation of thermal requirements for different life processes which characterize Pacific salmon. Freely compiled by reference to Donaldson and Foster, 1941; Brett, 1952; Brett and Alderdice, 1958; Seymour, 1956; Annual Reports of the International Pacific Salmon Fisheries Commission.

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Decomposition of Short-Chain Dicarboxylic Acid Esters during Separation on Polyester Gas Chromatography Media

SIR: The oxidative fission of polyethylenic fatty acids may yield a wide range of dicarboxylic acids which can be analyzed conveniently by gas chromatography of the methyl esters. Studies in this laboratory are being carried out with the homologous series oxalic acid to azelaic acid. Initial analyses carried out on gas-liquid chromatography columns employing silicone grease as the partition medium were satisfactory, but when polyester media were employed the two lowest members of the series failed to give quantitative results. This revelation is of extreme importance in determining the structure of polyethylenic fatty acids, which may yield a high proportion of these particular dicarboxylic acids on oxidative fission, and may also apply to analyses of other strong organic acids.

The problem was studied initially with a known mixture of dimethyl malonate, dimethyl succinate, and methyl undecanoate, the latter serving as a stable standard. The apparatus was a Podbielniak Chromacon, fitted with two columns. One was $^1/_4$ inch in outside diameter, 10 feet in length, and packed with 30% Craig succinate-butanediol polyester on 60- to 80-mesh Chromasorb. The other was $^1/_{16}$ inch in outside diameter, 8 feet in length, and packed with 30% Dow-Corning silicone grease on 40- to 60-mesh Chromasorb. The carrier gas was hydrogen and the flow was adjusted where possible to keep the retention times similar at various temperatures.

The results (Table I) show that the proportion of dimethyl malonate declined rapidly with increasing temperature when the polyester column was employed, but remained virtually constant on the silicone grease column. The ratio of dimethyl succinate to methyl undecanoate remained relatively constant on the polyester column, despite the longer retention time of the dimethyl succinate compared to dimethyl malonate, but the slight decrease may indicate some reaction. Dimethyl oxalate also showed appreciable decomposition, but as the retention time is short the results were less noticeable. Ethyl esters of oxalic and malonic acids decompose readily under the same conditions on polyester media.

Table I. Proportion of Methyl Esters Unchanged by Area (Uncorrected)

Temp., °C.	Polyester Column*		Silicone Grease Column*	
	Succinate	Malonate	Succinate	Malonate
140	0.98	0.63	0.78	0.71
150	0.93	0.40	0.86	0.76
160	0.92	0.22	0.90	0.70
170	0.86	0.11	0.90	0.70
180	0.87	0.02	..	..
190	0.84	0.00	..	..

* Value for undecanoate was 1.00 at all temperatures listed.

As the proportion of dimethyl malonate passing through the polyester column declined, a number of ill-defined peaks appeared near the injection point. When nitrogen was the carrier gas, severe negative deflections appeared in this region, indicating the presence of polar compounds. Use of a $^1/_4$-inch column, 10 feet in length, and packed with 10% LAC-3-R-728 polyester (succinate–diethylene glycol), did not significantly alter the proportion of oxalic and malonic esters decomposed, but did give much better resolution of the decomposition product peaks. Tentative identifications based on retention values indicated the presence of carbon dioxide, ethyl alcohol, and acetic acid in the case of diethyl malonate, and the presence of carbon dioxide, methanol, and acetic acid with dimethyl malonate. A number of other peaks could not readily be identified. Oxalic esters showed peaks corresponding to carbon dioxide and the alcohol, but formic acid could not be identified with certainty.

Methyl esters of weaker long-chain aliphatic acids interact with polyester partition media at somewhat higher temperatures (*3*), while the polyesters themselves may also be unstable (*1*). Because oxalic and malonic esters are thermally stable, the initial step is probably interaction with the polyester with liberation of the alcohol and the free acid, the latter then undergoing thermal decomposition to give carbon dioxide and a monobasic acid (*2*).

The possibility that the copper tubing employed in the columns caused this reaction was eliminated when stainless steel tubing packed with polyester media gave similar results.

Thermal degradation of a polyethylene glycol partition medium has recently been reported to give misleading analytical results through interaction of alcohol samples with the formic acid produced (*4*).

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FRB No. 629

CONTAINMENT OF RADIOACTIVE WASTE FOR SEA DISPOSAL AND FISHERIES OFF THE CANADIAN PACIFIC COAST

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CONTAINMENT OF RADIOACTIVE WASTE FOR SEA DISPOSAL AND FISHERIES OFF THE CANADIAN PACIFIC COAST

Abstract

Low-level radioactive waste consists largely of contaminated laboratory trash, larger items of equipment, animal carcasses from biological tracer experiments, active liquids, and some reactor-irradiated materials.

A total of 16,288 55-gallon drums of low-level waste has been dumped off the coast of California from 1946 to 1957, inclusive.

The major objectives of the criteria for US sea disposal operations have been (1) safety in handling between waste originator and disposal site, and (2) adequate sinking of wastes in the sea. There has been no requirement for integrity of container or contents at depth.

Other techniques used or previously suggested for radioactive waste containment, such as canning, adsorption on clay, and integration into glass are described. In order to render a radioactive waste harmless to fish and other aquatic organisms, it must be either (1) isolated from the environment, or (2) dispersed to levels of permissible concentrations. A spherical vault of suitable engineering design, hermetically sealed and able to withstand high pressures, is proposed for low-level and intermediate-level solid waste disposal, as a means of isolating the waste from the environment.

Fish may be affected by radioactive waste in their environment through: (1) direct radiation from the disposed radioactive material; (2) ingestion of food organisms containing concentrated radioisotopes; (3) irradiation by water containing radioactive ions or particles; and (4) contamination by bottom materials rich in precipitated radioisotopes.

Research necessary before widespread marine disposal of radioactive waste should be permitted is suggested.

LA MISE EN RECIPIENTS DES DECHETS RADIOACTIFS EN VUE DE LEUR ELIMINATION DANS LA MER ET LA PROTECTION DES PECHERIES OPERANT AU LARGE DE LA COTE CANADIENNE DE L'OCEAN PACIFIQUE

Résumé

Les déchets de faible radioactivité sont constitués surtout par des détritus contaminés provenant des laboratoires, des accessoires d'assez grande dimension, des carcasses d'animaux ayant servi à des expériences biologiques avec traceurs, des liquides radioactifs et certains matériaux irradiés provenant de réacteurs.

De 1946 à 1957 inclusivement, on a immergé, au large de la côte de Californie, un total de 16.288 bidons de 230 litres environ, remplis de déchets à faible radioactivité.

Au cours de ces opérations d'évacuation dans les eaux des Etats-Unis, on s'est efforcé d'observer deux critères principaux: 1) assurer la sécurité des manipulations entre la source des déchets et le lieu d'évacuation et 2) d'engloutir de façon appropriée les déchets dans la mer. Aucune condition n'a été fixée au sujet de l'intégrité du récipient ou du contenu, une fois parvenus dans les profondeurs de la mer.

Le mémoire expose d'autres méthodes pour renfermer des déchets radioactifs qui ont été utilisées ou suggérées précédemment: mise en récipients métalliques, adsorption sur de l'argile et intégration dans du verre. Afin de rendre les déchets radioactifs inoffensifs pour les poissons et autres organismes aquatiques, il faut 1) soit les isoler du milieu environnant, 2) soit les disperser afin d'abaisser leur radioactivité à la concentration autorisée. Il a été proposé, pour isoler les déchets du milieu environnant, de les placer dans un récipient sphérique construit selon un plan approprié, scellé hermétiquement et capable de résister à de hautes pressions; il servirait pour l'élimination des déchets solides à radioactivité faible ou moyenne.

Les poissons peuvent être affectés par les déchets radioactifs de plusieurs manières:

1) par rayonnement direct des matières radioactives éliminées;
2) par ingestion d'organismes alimentaires contenant des radioisotopes concentrés;
3) par irradiation de l'eau contenant des ions ou particules radioactifs et
4) par contamination, au contact de matières riches en radioisotopes précipitées en profondeur.

L'auteur pense qu'il est bon de poursuivre les recherches avant d'autoriser l'élimination en grand des déchets radioactifs dans la mer.

УДАЛЕНИЕ РАДИОАКТИВНЫХ ОТХОДОВ В МОРЕ В КОНТЕЙНЕРАХ И РЫБОЛОВСТВО В КАНАДСКИХ ПРИБРЕЖНЫХ ВОДАХ ТИХОГО ОКЕАНА

Резюме

Отходы с низким уровнем радиации состоят главным образом из загрязненных лабораторных отходов, более крупных частей оборудования, трупов животных после биологических экспериментов с меченными атомами, активных жидкостей и некоторых материалов, облученных в реакторе.

За период с 1946 года по 1957 год с берегов Калифорнии было сброшено отходов с низким содержанием радиоактивных веществ в количестве 16288 55-галлонных бочек.

Главными критериями для Соединенных Штатов при удалении радиоактивных отходов в море являются: 1) техника безопасности при перевозке отходов от их источника до места их удаления и 2) соответствующие условия при погружении их в море. Никаких требованиий относительно целостности контейнеров и их содержимого по погружении на глубину не предъявляется.

Дается описание контейнеров, которыми пользуются или которые раньше предлагались для заключения в них радиоактивных отходов, например: запаенные металлические коробки, адсорбция глиной и вплавка в стекло. Для того, чтобы сделать радиоактивные отходы безвредными для рыб и других водных организмов, надлежит 1) либо изолировать их от среды, 2) либо разбавить радиоактивные отходы до уровня допустимой концентрации. Предлагается для удаления твердых отходов с низким и средним уровнем радиоактивности применять сферический резервуар, соответственно сконструированный, герметически закупоренный и способный выдерживать высокие давления, как средство изолирования отходов от морской среды.

Радиоактивные отходы могут оказать воздействие на рыбу в морской среде через:

1) прямое облучение удаленными радиоактивными материалами;
2) заглатывание организмов, служащих пищей для рыб, содержащих концентрированные радиоизотопы;
3) облучение водой, содержащей радиоактивные ионы и частицы; и
4) заражение донными материалами, имеющими высокое содержание осевших на дно радиоизотопов.

Выражается мнение, что прежде, чем может быть разрешено удаление в море радиоактивных отходов, необходимо произвести надлежащие исследования.

CONFINAMIENTO DE DESECHOS RADIACTIVOS PARA SU EVACUACION EN EL MAR, EN RELACION CON LAS PESQUERIAS DE LA COSTA CANADIENSE DEL PACIFICO

Resumen

Los desechos de baja radiactividad suelen ser, por lo general, basuras de laboratorio contaminadas, piezas de equipo, cadáveres de animales sometidos a experimentos biológicos con trazadores, líquidos activos y materiales de reactor irradiados.

Desde 1946 a 1957, inclusive, se sumergieron frente a la costa de California 16.288 toneles de 230 litros de capacidad cada uno que contenían desechos de baja actividad.

Los criterios seguidos por los Estados Unidos en las operaciones de evacuación de desechos en el mar responden a dos objetivos principales: 1) Conseguir que la manipulación de desechos entre el punto de origen y el de evacuación se efectúe en las mejores condiciones de seguridad; 2) hundir los recipientes a suficiente profundidad. No se ha establecido ningún requisito por lo que se refiere a la integridad del recipiente o del contenido después de la evacuación.

El autor describe otras técnicas utilizadas o sugeridas para el confinamiento de desechos radiactivos, como el envasado en latas, la adsorción a arcilla y la integración en vidrio. A fin de que los desechos radiactivos resulten inocuos para los peces y demás organismos marinos, es preciso aislarlos del medio o dispersarlos hasta que su grado de concentración no exceda del valor máximo permisible. Para aislar del medio a los desechos sólidos de baja y mediana actividad evacuados, el autor propone que se emplee un recipiente esférico de apropiado diseño mecánico, herméticamente cerrado y capaz de resistir grandes presiones.

Los peces pueden sufrir de cuatro maneras diferentes los efectos de los desechos radiactivos que hay en el medio: 1) por irradiación directa de las sustancias evacuadas; 2) por ingestión de organismos que contienen radioisótopos concentrados; 3) por irradiación debida a las partículas o iones radiactivos contenidos en el agua; 4) por contaminación con materiales del fondo ricos en radioisótopos precipitados.

El autor sugiere las investigaciones que será necesario efectuar antes de permitir la evacuación en gran escala de desechos radiactivos en el mar.

CONTAINMENT OF RADIOACTIVE WASTE FOR SEA DISPOSAL AND FISHERIES OFF THE CANADIAN PACIFIC COAST

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1. Introduction

Radioactive wastes of low-level activity in the United States originate in tracer laboratories of universities and various private and government agencies licensed by the United States Atomic Energy Commission (USAEC) to use radioisotopes. The same type of laboratories contribute small quantities of low-level waste in Canada.

Early in the era of tracer research, these wastes were generally eliminated by being contained in a suitable trash can, ballasted with concrete or other heavy substance and released at sea some distance from shore. This practice commenced about 1946 as a garbage-disposal type of operation without close control or regulation. As these operations increased, some control began to be exercised with improvements in packaging methods and dumping procedures. Records have been maintained of the number of containers of radioactive waste dumped and the general location of the dumping area. Estimates of curie content of packages are very rough, based on the judgement of individuals, and can be in error by as much as a factor of 10.

It is obvious that wastes of this type are most easily eliminated by appropriate packaging and dumping into the sea at a safe distance from shore. The fact that no one laboratory produces a large amount of low-level waste, that laboratories producing small amounts of such waste are numerous and widespread, and that most such laboratories are located some distance inland from the sea eliminates the consideration of a central disposal centre where wastes could be appropriately treated and effluents could be suitably discharged.

Some of the background information on and present status of regulations, licensing and disposal of low-level radioactive wastes in the United States is given in a statement presented to the State of California Senate Interim Committee on Fish and Game (USAEC, 1958) (1). The data on Pacific coast sea disposal of low-level radioactive wastes to 1956 taken from JOSEPH, A.B. (2a and b) are given in Tables I and II.

A major problem in disposal of packaged radioactive waste off the Pacific coast of North America is its possible interference with the fisheries. The bottom-dwelling fish would be the ones most likely to be affected by diffusion of radiomaterial from the packages and/or by precipitation of radioisotopes on the sea bed. Moreover, accumulation of containers on the sea bottom can be a hazard to trawl gear.

It is assumed that the exchange between bottom water and the surface, wind-mixed layer is slow enough so that all the short-lived isotopes and many of the longer-lived ones will not be transported or diffused from the bottom to the surface in significant concentrations. Consequently, the highly migratory fishes, such as the salmon and tuna, will not be considered here.

TABLE I

YEARLY SUMMARIES OF RADIOACTIVE WASTES DUMPED IN THE PACIFIC OCEAN BY THE UNITED STATES (2a)

A. OFF SAN FRANCISCO, CALIFORNIA

Item	1946—1951	1952	1953	1954	1955	1956	Total
1. Number of 55-gallon drums	3,000	1,132	1,448	2,100	2,300	2,600	12,580
2. Number of concrete blocks	19	12	19	36	29	35	150
3. Estimated curie content			*				10,000

* Estimated 30 curies of long-lived alpha activity dumped from 1946 through 1953.

B. OFF SOUTHERN CALIFORNIA

Item	1953	1954	1955	1956	Total
1. Number of 55-gallon drums	103	85	271	370	829
2. Estimated curie content	<1	10	30	<1	42

TABLE II

SUPPLEMENT YEARLY SUMMARY OF RADIOACTIVE WASTES DUMPED IN THE OCEAN BY USAEC AND DOD (2b)

A. OFF SAN FRANCISCO, CALIFORNIA*

Category of waste	Nature & extent of contamination	1956—1957	
		(55-gallon drums)	
Combustible solids	α, β, γ; $T^1/_2 > 1$ yr; 2—10 mc/pkg.	2,332	2,636
Solidified liquids**	F.P.'s + Transuramics; 3.3—166 c/pkg.	56	64
Contaminated glove boxes	Various; 3—10 c/ca.	($\sim 5' \times 5' \times 8'$)	
		67	72
Low-level liquids		190	179

B. OFF SOUTHERN CALIFORNIA

Category of waste	Nature & extent of contamination	1956
		(55-gallon drums)
Solids (all)	10.19 mc of Th, U; between 3 & 29 c.	855
Solidified liquids	Unknown isotopes -total	25

* Does not include radioactive wastes from other Government agencies in areas which also use NRDL service.

** Dry mixture, measured volume of vermiculite and concrete. For high levels the drum is encased in concrete block.

From the few deep-water explorations by scientific vessels, it is known that many forms of invertebrate and fish fauna exist at great depths. As fisheries explorations move into deeper water, there is growing evidence that certain commercial fish species can be found at greater depths than previously recorded. Little is known of the effects that packaged, low-level, radioactive wastes would have on these bottom fish and on the organisms on which they feed.

The problem concerning low-level radioactive waste disposal on the east coast of the United States has been well summarized, with specific recommendations, in Publication 655 of the National Academy of Sciences (3). The shortcomings of existing knowledge and needed research are fully stated in Chapter 5 of *Oceanography* 1960 *to* 1970 (4) 1959 b.

2. History of radioactive waste disposal off the Pacific coast of North America

To date, the wastes dumped into the Pacific from North America have originated largely from Californian laboratories. These have not included as many packages of reactor irradiated materials as the Atlantic disposal. However, a greater number of drums containing low-level liquids were included in the California wastes than in those from the East Coast. Clean-up type material evolving from operating particle accelerators and containing mainly alpha-emitting wastes constituted the contents of many of the first drums released into the Pacific. An estimated 30 curies of alpha activity had been dumped by the end of 1953.

2.1 *Areas of radioactive waste disposal* 1946—1956

Radioactive wastes from US Pacific Coast operations have been dumped mainly in three areas of the Pacific (see Fig. 1).

2.1.1 Off Southern California

Dumping since 1953 has occurred 60 miles off the Southern Californian coast in a 1,000-fathom deep "pot hole" in the Santa Cruz basin (33° 39′ N; 119° 28′ W). These dumping operations have been carried out by the US Navy at the request of USAEC.

2.1.2 Off Central California

The area south of the Farallon Islands, west of San Francisco, has the longest record on the Pacific Coast of receiving radioactive wastes. At the original site, 22 miles from San Francisco in a depth of about 50 fathoms, three tug-loads of radioactive wastes were dumped in 1946. Later that year, the dumping location was changed to a point 38 miles west of San Francisco in 1,000 fathoms of water. This was used until 1951 when a point 30 miles from San Francisco on the 500-fathom contour was selected for dumping. Since January 1954, dumping has been resumed at the 1,000-fathom line. Occasionally, when sudden inclement weather arises or because of malfunctioning equipment, radioactive wastes have been deposited in other than the designated areas. Dumping has probably not occurred outside the area delineated by the dashed lines in Fig. 1. At present, vessels carrying radioactive waste for disposal at sea do not leave port under adverse weather conditions. Position of disposal is closely controlled by Loran or Shoran navigational aids.

Fig. 1
Chart of the Pacific Coast of North America showing locations of disposal sites used to 1958 and new sites proposed in 1958 for low-level radioactive wastes

2.1.3 Off the US Pacific Northwest and British Columbia

Records of waste disposal from the US Pacific Northwest and from British Columbia are scanty. Several drums of waste from radioisotope tracer experimentation at the University of Washington have been taken out by the University's oceanographic vessel, M. V. *Brown Bear*, and dumped in the ocean off the Washington coast. At least one instance of sea disposal of radioactive wastes from the University of British Columbia is known to the writer, who assisted with such disposal from the Canadian Navy's oceanographic vessel, HMCS *Cedarwood*, in March, 1950. More recent disposals have been carried out at a depth of 1,000 fathoms by the Pacific Naval Laboratory, Esquimatt, B.C., with the waste incorporated into concrete blocks (Fleischer, personal communication).

3. Categories of low-level radioactive waste

Most of the wastes considered here consist of a heterogeneous collection of contaminated laboratory trash. Paper wipes, blotting paper, rags, maps, ashes, broken test tubes, beakers and other contaminated laboratory glassware constitute a large proportion of this waste. As well, there are contaminated laboratory equipment, such as air filters and duct works, and animal carcasses used in tracer and other biological experiments. Some liquids of activity of the order of microcuries per litre must be packaged and dumped.

3.1 *Combustible solids*

Materials which are organic in nature and usually contaminated by mixed fission products or other beta-gamma emitters having half-lives greater than one year fill this category. Radioactivity contained in drum packages of these materials can range from 0 to 1,000 mc, but the average is about 50 mc. Radiation of up to 500 milliroentgens per hour has been measured at the surface of such packages.

3.2 *Other contaminated solids*

This category consists of contaminated laboratory equipment, such as damaged air ducts and used air filters. Usually these items have been contaminated by beta-gamma emitters with half-lives greater than one year. Radioactivity of such material may range as high as 30 curies per drum, with a median figure of about 200 mc per drum. Packages of such wastes can register as high as 1 roentgen per hour at their surface.

3.3 *Reactor-irradiated materials*

Included in this category are reactor fuel samples and other reactor experiment materials and by-products of isotope production. These materials have relatively high specific activities. The space in drums used for holding such wastes is largely taken up by lead and concrete shielding. The radioactivity contained by one package is usually limited to about 10 curies. Emitted radiation at the drum surface may be as high as 1 r per hour.

3.4 *Soldified Liquids*

Low-level and intermediate-level liquid wastes are classified under this category. These wastes are incorporated in cement mixtures or with chemical gelling materials before packaging and dumping. Drum packages of these wastes may contain up to 100 mc of radioactivity with an external radiation of as high as 1 r per hour.

4. Regulations in radioactive waste disposal

In packaging wastes, the major objectives of criteria for sea disposal operations are given as follows by USAEC:

4.1 *Adequate protection for handling*

Packaging must be adequate for safety in handling between the site of the waste originator and the final disposal. The radiation limits are 200 mr/hr. at the surface and 10 mr/hr. at one metre from the source. The package must be so constructed as to withstand handling operations during loading and unloading procedures without exposing the contents. It must be properly labelled, and if materials are shipped by common carrier, conformity with the Interstate Commerce Commission and Coast Guard regulations is required.

4.2 *Satisfactory sinking in the sea*

To insure that packages sink throughout the whole water column in the sea, the packages must have a minimum of 10 lb/gal of displacement volume. While a package is not required to be so constructed as to maintain complete structural integrity at 1,000 fathoms, it must be prepared in such a way that parts of it or its contents will not float to the surface should the container rupture due to pressure.

Regulations are spelled out more fully in Appendix III, "Packaging of Contaminated (Radioactive) Scrap for Disposal", of the CARRITT Report (5).

5. Packaging methods for radioactive waste disposal

The most common technique employed in the United States for packaging low-level radioactive waste has been containment in standard 18-gauge, 55-gallon drums with concrete for ballast and shielding. For the larger items of contaminated equipment, concrete vaults have been fabricated. The various adaptations of the drum and concrete vaults for packaging radioactive wastes are described and illustrated in Appendix II, "A Special Report on Disposal of Radioactivity into Atlantic Ocean Waters Past, Present and Predicted", of the CARRITT Report (5), and in "United States Sea Disposal Operations—A Summary to December 1956" (2a).

Other techniques for radioactive waste disposal have been suggested where dangerous fission products can be fixed into a solid medium and thereby isolated from the environment. These have been designed largely for land disposal as a means of overcoming the hazards of storage of high-level wastes in tanks, or seepage of the waste into the ground.

5.1 *Adaptation of 55-gallon drums to waste disposal*

Second-hand drums, reconditioned with a coat of paint in some cases, have been used throughout. Drum containers are used primarily because they are: (a) readily available, (b) inexpensive, (c) quite easily handled, and (d) versatile enough so that they can be adapted in various ways for suitable shielding with concrete and lead for protection in handling. Some of the various methods for utilizing these drums for waste disposal are given in the following:

5.1.1 Bales of compressed combustibles containing radioactive wastes are inserted into a drum and concrete is poured into the space between the bales and the walls of the drum.

5.1.2 The radioactive waste materials are mixed with concrete into a space in the drum between a base and cap of concrete.

5.1.3 A 30-gallon drum of ashes or solidified liquids is inserted into the 55-gallon drum and concrete poured around it.

5.1.4 A preformed concentric cylindrical space can be built into the concrete, lining a drum for the more highly radioactive wastes. This space is filled with the active materials and a concrete cap is poured on it.

5.1.5 A preformed, concentric, cylindrical space lined with lead has been used for the irradiated and relatively high-level radioactive wastes.

5.1.6 Low-level liquids have usually been solidified by mixing into concrete or by gelling with certain chemical compounds. These solids are confined in drums lined with concrete.

5.1.7 In some instances, a glass carboy of low-level waste is placed on a slab of concrete in a drum and fresh concrete is poured into the space between the carboy and the drum wall.

5.2 *Concrete vaults for waste disposal*

5.2.1 The "glove box package" has been used solely in disposal off San Francisco. It is a rectangular box with outside dimensions: 8 ft long by 5 ft wide by 7 ft high. A 6-inch, wire-mesh reinforced bottom is poured first. Then the side walls are constructed of reinforced concrete and the wastes are inserted. Concrete is poured to the top level of the walls. The mesh-reinforced walls and top are a minimum of 6 inches thick. A vent through the upper concrete slab to the interior allows pressure equalization to take place between inside and outside.

Such concrete boxes usually contain from 2 to 10 curies of activity each. Several are known to have contained as much as 500 curies.

5.2.2 A small-size burial vault has been used as an expedient package by an east coast installation. It has interior dimensions of 40 in by 16 in by 16 in with a capacity of 5.9 ft^3. The bottom is $3^1/_2$ in thick and walls 2 in thick. It weighs empty 600 lb and loaded 900 to 1,300 lb. A steel lifting cable secured firmly into the concrete facilitates handling.

5.3 *Canning of radioactive wastes*

A scheme used in canning highly-active sludge from the treatment of radioactive wastes at the Atomic Weapons Research Establishment, Aldermaston, England, has been described by Wilson *et al.* (6). The basic requirements in this technique were:

1. Sludge was to be discharged into cans, which would be sealed and dumped into the sea.
2. The cans were to be strong enough to withstand even abnormally rough treatment without leaking.
3. Operators were to be completely protected during the entire operation of filling and sealing the cans.

A five-gallon can (Imperial measure) was considered to be the largest which could be handled conveniently. A prototype was designed accordingly with 3/16 in mild steel wrapper plate and 1/4 in ends. The neck piece was provided with a protecting shroud and was arranged to be closed with a rubber bung and externally screwed-on steel cap. As a precaution against opening

the cap once the can was filled, a hole was provided in the top of the cap to take a hardened steel pin. When rammed home, the pin destroyed the thread, making subsequent opening impossible.

Canning offers little hope of packaging anything except sludge or liquid because of the effect sea pressure would have on the can should combustible solids or other compressible trash be so contained.

5.4 *Adsorption of radioactivity on clay*

Clay has been shown to be an excellent medium for adsorbing radioactivity from solutions. Fission-product cations are selectively adsorbed by montmorillonite clay, which has a high exchange capacity of 1.12 milliequivalents per gramme [GINELL *et al.* (7)]. The layer-lattice structure with the exchangeable ions located between the lattice layers of this mineral allows the strong adsorption of cations. Adsorbed radioactive ions can be sealed within the crystal lattice by heating the clay to 1,000° C. Montmorillonite clay is highly stable before and after heating and following radiation exposure. In addition to these qualities, montmorillonite clay has the advantage of being widely available at relatively low cost.

It has been suggested at various times [HATCH (8); AMPHLETT and WARREN (9)] that high-level liquid wastes can be passed through columns of montmorillonite clays for the removal of the radioactivity. Following suitable preliminary treatment, the wastes could be passed through a bank of ion exchange columns where the water passing through the clay would come out relatively free of radioactivity. The radioactive clay can be consolidated by a kiln process where it is fired at 1,000° C to produce high-density, high-specific-activity, insoluble bricks. Short-term stability tests suggest that the montmorillonite clay bricks would be extremely stable. It is unknown what the behaviour of the bricks would be in sea-water at great depths.

While the scheme is feasible, it has several disadvantages:

(a) It is only suitable for liquid wastes where non-active ions have been removed by pre-treatment and the radioactive ions have been concentrated.

(b) Ruthenium-106 is not removed by this exchange process.

(c) While a cost estimate of the process on an industrial scale has not been examined, it is believed that the operation would be costly. There would be required a central agency to carry out the clay adsorption and baking process for the numerous laboratories producing intermediate-level and low-level liquid wastes. Hazards involved in handling unprotected radioactive bricks would necessitate special handling equipment and shielded containers for shipping.

5.5 *Fusion of radioactive waste in glass*

Integration of radioactive waste into glass has been investigated at Chalk River, Canada, as a means of storing highly-active liquids in a solid form [WATSON *et al.* (10)]. Nepheline syenite, commonly used in the glass and pottery industry, is mined in an unusually uniform deposit in Ontario and is available as a finely-ground powder. Mixed with 15% CaO as a flux and fused at 1,350° C, nepheline syenite was found to incorporate many of the radioactive constituents from reactor wastes. The glass formed on cooling was found to have a high resistance to leaching.

One of the problems encountered in this method was that ruthenium and

caesium are volatilized during the fusion process. It is necessary to remove these elements from the gas stream by a scrubber charged with iron oxide at 400 to 800° C. It seems hardly an economical disposal technique for processing low-level radioactive liquids since costs are estimated at between $ 1.6 and $ 2.5 per litre.

While disposal of the radioactive glass in the ocean as a final burial place is an attractive proposition from the point of view of adequate storage space and good dissipation of heat from radioactive decay, release of activity by leaching from unprotected glass would be high. No experiments have been reported on containment of the glass to reduce leaching in water.

6. Behaviour of radioactive waste containers used to date

Not all containers used for marine disposal of radioactive waste have undergone rigid tests at sea. However, there are some results of tests on standard drum containers. No results of tests have been reported on concrete vaults.

6.1 *Drum containers*

Tests of drum containers in July 1958 by the California Department of Fish and Game (11) showed that no damage occurred when the drums were lowered into the sea so long as the interior of the drum was exposed to pressure equalization from the outside.

A drum container partially filled with trash and ballasted to 600 lb with gravel was sealed with a lid fitted with a rubber gasket and bolted down water-tight. This container collapsed so that the lid seal was broken at less than 100 fathoms. None of the solid contents of the container was released.

In three representative drums, concrete was used to enclose the contents. The concrete mix consisted of 1 part cement to 3 of sand and $3^1/_2$ of gravel. The concrete was poured from a concrete-mix truck. The bottom layer was allowed to cure for six days before the walls and cap were poured. The final pour was cured for five days before testing.

One drum contained a 5-gallon paint can surrounded by concrete. It was connected to the outside by a soft copper tube open at the outside end and flattened inside the can and folded over to act as a controlled pressure-release inward. When tested to 400 fathoms, the copper tube burst open as planned and no damage was sustained by the container.

Two drums were set up with open cavities lined by concrete inside the drum and no connection to the outside for pressure equalization. In one drum, concrete was poured on the bottom to a depth of 8 inches and 6 empty cylindrical ice-cream cartons of $2^1/_2$ gallons capacity each were placed one on top of another as centrally located as possible and fastened in place with wooden battens. The remainder of the void was filled with concrete. This barrel was tested to a depth of 400 fathoms. Apparently a violent implosion crushed in one side rupturing the metal along lines where no seams were previously present.

The second drum with the sealed-off space inside consisted of a 5-gallon paint can centrally enclosed in concrete. When tested to a depth of 400 fathoms, this unit collapsed, a cylindrical hole forming in the 6 in. concrete over the end of the paint can equal in diameter to the can.

Recent tests have also been carried out by the Scripps Institution of Oceanography on drums of the type used for radioactive waste disposal. These all

collapsed within the upper 200 fathoms (Isaacs, John D., personal communication).

A metal drum, although usually painted on the outside, cannot for long withstand the corrosive action of sea-water. Its life expectancy is probably no more than 10 years in the sea, as calculated by Joseph [(5) Appx. X]. The concrete used in encasing the radioactive waste is probably not very consistent and often of poor quality. A normal-type concrete is said to survive, on the average, 100 years with exposure to the atmosphere. The action of sea-water would probably reduce this survival time by 2/3 to about 30 years. It can be assumed that the concrete holding the radioactive contents of the drum will not maintain its identity for more than 30 years.

6.2 *Can containers*

The prototype of the can used for radioactive sludge in England was tested hydraulically to 100 lb/in^2 and was then dropped from a height of 20 feet on to spiked angle irons (6). Only a slight distortion of the neck shroud resulted, the body and cap remaining sound and undamaged. The performance of these cans at sea was not described. A can such as this may or may not remain intact at pressure exerted at 2,000 fathoms where there is a pressure of about 6,000 lb/in^2. Certainly corrosive action of sea-water would render metal cans rather short-lived.

7. A proposed container for radioactive waste disposal

There are two alternatives for safe disposal of radioactive waste. First, the radiomaterial can be isolated from the environment to insure that at some future date dangerous fission products do not become free chemical agents available to take part in the life-cycles of plants and animals. Secondly, the radioactive wastes can be dispersed into the environment to concentrations at tolerable levels. The type of materials considered in low-level waste makes controllable dispersion difficult. Consequently, one must turn to suitable containment.

One of the uncertainties in low-level radioactive waste disposal can be eliminated if a scientifically-engineered container is devised. This container could be designed so that its behaviour in the sea is known. It should be hermetically sealed and reinforced to withstand pressures at 2,000 fathoms without rupture. While design and development of such a container would be confronted by various technical difficulties, the ultimate production of a satisfactory prototype should not be impossible.

A concept of the type of package proposed is shown in Fig. 2. It was designed, after consultation on this problem with the writer, by Mr. Arthur Robinson. The vault would consist essentially of a sphere of dense aggregate concrete holding a dense glass container surrounded by lead shielding. Enclosing the concrete would be a polyethylene shroud. A dense glass lid would enclose the radioactive wastes followed by a tapered concrete manhole cover and a screw-on shroud lid. Adequate shielding would provide protection in handling during transport from the source of waste to the disposal site. Rings set into the concrete would facilitate handling.

This container would essentially fulfil the requirements of the most active type of waste considered here, as given in section 3.3. It could be a suitable replacement for the type of drum container described in section 5.1.5. The vault would have to be so designed as to reduce leaching of the radioactive

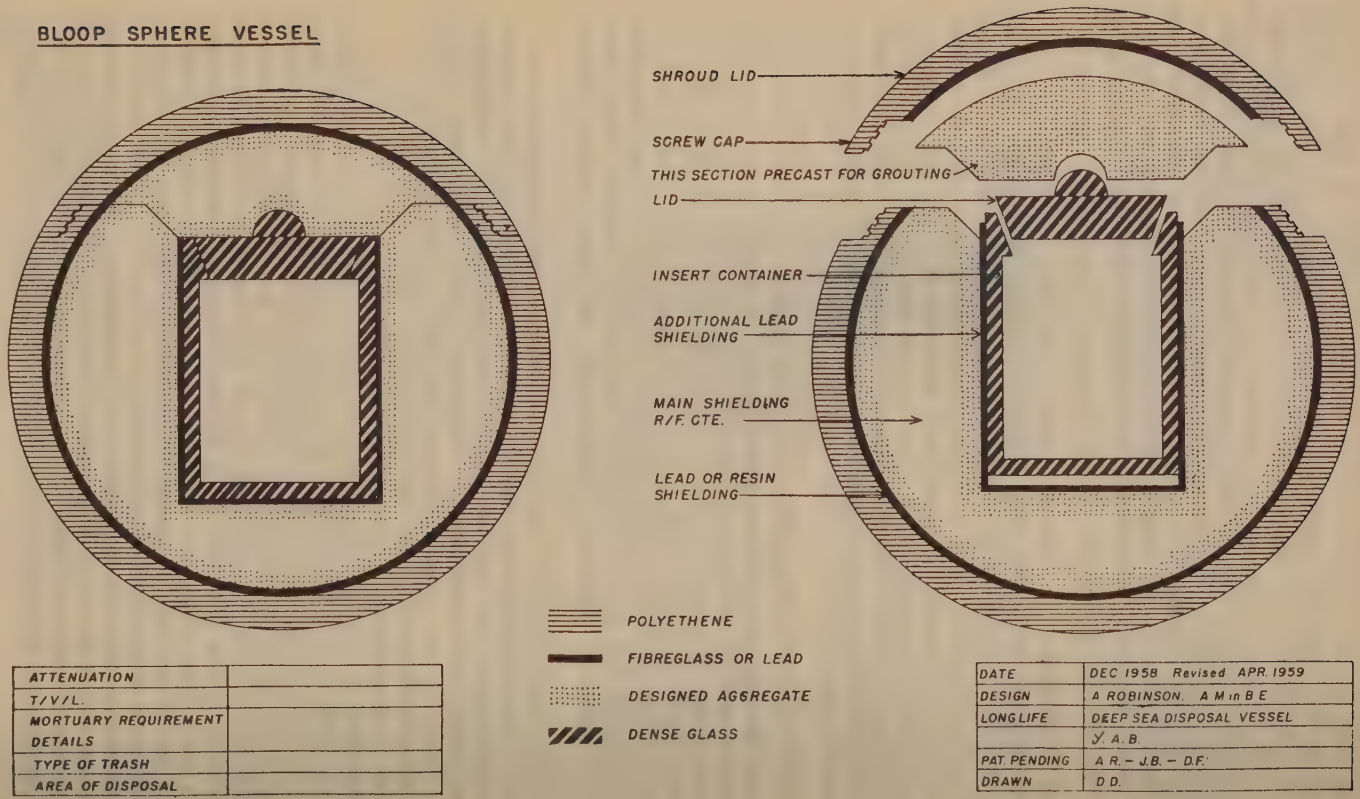

Fig. 2
A pressure vesse proposed for sea disposal of radioactive waste

material to a minimum and withstand pressure up to 9,000 lb/in^2 (pressure at 2,000 fathoms plus a 50% safety factor).

7.1 *Advantages of properly-designed container*

There are a number of obvious advantages to a standard prefabricated vault which meets certain minimum specifications in shielding, strength, and containment.

7.1.1 Supervision by regulatory bodies would not have to be nearly as great, inasmuch as the requirements of (a) safety in handling (i.e. maximum permissible external radiation, no exposure of contents during loading, transit, and unloading, proper labelling) and (b) rapid sinking in the sea, would be automatically met.

7.1.2 No preparation of concrete for ballast and shielding would be required by the originator of the waste or the disposal agency. Work in packaging by the laboratory contributing the waste would be reduced to a minimum.

7.1.3 With a well-established behaviour of a standard vault, through thorough testing in the field, a better estimate can be made of the radioactivity in the water caused by irradiation leaching, etc. from the contents.

The life expectancy of a specially-prepared, dense-aggregate concrete, as it is proposed for a well-designed vault, would be of the order of 1,000 years. In that time, all of the short-lived and most of the intermediate and long half-life radioisotopes would have decayed.

7.1.4 A properly-designed container might offer possibilities of other means of sea disposal than discharge at depths of 2,000 fathoms. It is conceivable that burial of the vaults could be achieved by allowing them to penetrate into marine mud found in certain basins, some of which lie on the continental shelf close to the land [Renn (2)]. Further research on this type of burial is necessary.

8. Disposal of packaged radioactive waste and possible bearing on the fisheries off the coast of British Columbia

If it is assumed that all packaged radioactive waste ultimately reaches the bottom of the sea and that dispersion and leaching of the radioactive material from the container during its descent from the surface to the bottom is negligible, then we can confine ourselves to the consequences to bottom organisms. This further pre-supposes that exchanges between the deep water and the upper, mixed layer is slow enough that contamination of the surface water by the deposited wastes is negligible. The highly migratory fishes, such as salmon and tuna, can therefore be ignored in the first approximation. However, the many commercial forms which live on or near the bottom deserve some scrutiny.

8.1 *Behaviour of bottom fish in relation to contamination by radioactive waste*

Compared to pelagic fish frequenting the upper layers of the sea, most bottom fish exhibit only small migrations. Certain of the cod-like ground-fish appear to be almost sedentary, occupying specific reef areas the year around. Many of the flounder-like flat-fish make limited migrations annually from

feeding grounds to their spawning areas. This apparent lack of extensive migration favours disposal of packages of radioactive waste in those areas where ground-fish are known to be absent. However, continuous exposure to radioactivity can be experienced by those fish which happen to be in the area chosen for radioactive waste disposal.

Some of the bottom fishes are known to seek out reefs, large boulders and any other type of prominent object for shelter and protection. These objects are not necessarily confined to natural outcrops but may also be derelicts of shipwrecks, discarded rubbish from ships, and other refuse. This means that barrels or other containers of radioactive waste discharged on to flat, muddy or sandy, unrelieved bottoms would be sought as havens of refuge by the round, or perch-like, bottom fishes. Because of their gregarious nature, these fishes would have a further tendency to congregate near prominent objects.

The behaviour of the flat-fishes is somewhat different. Unlike the rock-fishes, the flounder-type fishes seek flat, sandy bottoms. Contacts with containers loaded with radioactive waste on the bottom would be only accidental. A greater hazard would be radioisotopes precipitated on the bottom as a result of discharge of liquid wastes. While the dilution of the radioactivity by the sediments is great, inasmuch as the precipitation is spread over a large area, fairly constant contact of the bottom fish with the radioactive sediments is assured.

8.2 *Bottom topography*

The bottom topography has a great bearing on the ground fisheries in that distribution of the commercial stocks of bottom fish is related to depth and type of bottom. Topography is a primary consideration in radioactive waste disposal in that usually disposal sites are chosen where the water is deep, at a safe distance from the shore and removed from sea-mounts and submarine canyons.

The continental shelf off the coast of British Columbia is moderately broad in comparison to that off the Pacific coast of the United States, especially California. The fringe of islands, dominated by the Queen Charlottes and Vancouver Island, presents a 100-mile margin of relatively shallow, sheltered waters. Depths increase rapidly to 1,000 fathoms seaward of the continental slope. The change in depth thereafter, however, is gradual. A depth of 2,000 fathoms is reached at an average distance of 300 miles from the edge of the continental shelf. In contrast, the bottom off the coast of California drops off to depths of 2,000 fathoms at a distance of only 75 miles off the coast near San Francisco and 120 miles off Los Angeles, the major California areas from which radioactive waste disposal has occurred. (See Fig. 1).

The proposed restriction of radioactive waste disposal to a minimum depth of 2,000 fathoms off the Pacific Coast may meet with approval from waste dischargers along the California coast, who require only 75 to 120 miles of sea travel to dispose of their waste. However, objections may be raised by those individuals having the responsibility of disposing of radioactive wastes off the coasts of Oregon, Washington, British Columbia and Southeast Alaska, who may have to travel 300 to 400 miles to reach 2,000 fathoms.

It remains a problem that should be investigated, whether harm can be caused to the fisheries or other resources by disposing of radioactive wastes at depths between 1,000 and 2,000 fathoms off the BC coast. The additional

cost that severe restrictions impose on waste disposal because of lack of knowledge must be balanced in terms of the cost of investigations which might possibly relax the restrictions.

8.3 *Depth of trawl fishing*

The maximum depth to which bottom fish off the BC coast are fished at present is about 250 fathoms. Fisheries scientists express doubt that depths of trawling or long-line fishing off BC will ever exceed 400 fathoms. Most of the trawling at the present time is done on the continental shelf. Trawling in Hecate Strait is carried on at depths in the neighborhood of 40 fathoms. In the Strait of Georgia, trawling at depths greater than 50 fathoms seldom occurs. Off the mid-west coast of Vancouver Island, in the "Esteban Deep", where depths up to 250 fathoms are found, an extensive fishery for brill or petrale sole (*Eopsetta jordani*) has occurred [ALVERSON, CHATWIN (13)].

It is not the intention to suggest here that sizeable stocks of ground-fish will never be found at depths greater than 400 fathoms along the BC coast. Trawling explorations along the east coast of Canada have yielded catches of redfish (*Sebastes marinus*) in all sets from 150 to 500 fathoms, the largest catches being in the ranges of 176—200 and 301—350 fathoms [TEMPLEMAN, p. 123 (14)]. The redfish is a relative of the rockfish of the Pacific Ocean. Bottom fish have been found off the coast of California at depths in excess of 600 fathoms (Isaacs, John D., personal communication). It is conceivable that, in time, considerable numbers of bottom fish may be found off the BC coast at depths greater than 400 fathoms. However, trawlers may never find that fishing these stocks is profitable. On the other hand, we must face the possibility that future demands for fish and improvements in fishing vessels, gear and methods of preservation of fish may induce trawlers to go much further afield than envisaged at present.

9. Records of fishes taken in deep waters off the B C coast

One of the most valuable records of fish taken in deep water off the Pacific coast of Canada is that of the United States Fish Commission Steamer, *Albatross*. During 6 voyages between 1888 and 1906, this exploratory vessel made 43 dredge hauls in BC waters or off the coast. Depths of hauls ranged from 14 to 1,600 fathoms. Fish were obtained from 25 of these hauls and are listed by WILBY (15). A total of 55 species was captured, which represents roughly $^1/_4$ of the total known species in 1934. Some of the species from the *Albatross* collection have not been re-discovered for lack of deep trawling.

It is of interest that two small specimens of the big-scaled black smelt, *Bathylagus milleri*, were taken on 19 May 1906 at the *Albatross* Station 4,758 (52° 02′ N; 132° 21′ W) southwest of Moresby Island, at a depth of 1,600 fathoms. Since 1929, this species has been taken by the International Fisheries Commission at nine stations in the vicinity of the Queen Charlotte Islands at depths ranging from 220 to 488 fathoms [CLEMENS and WILBY (16)]. This is an indication that this species has a large vertical range. While the big-scaled black smelt may never be exploited commercially, it may transport radioactivity from contaminated deep water into the depth range commonly trawled.

Two specimens of the long-finned cod, *Antimora rostrata*, were taken by the *Albatross* on 29 August 1888 at Station 2,860 (51° 23′ N; 130° 34′ W) near

Cape St. James at a depth of 876 fathoms. The only subsequent capture of a specimen was also made by the *Albatross* on 3 September 1890 at Station 3,342 (52° 39′ N; 132° 38′ W) west of Moresby Island at a depth of 1,588 fathoms. It is noteworthy that this member of the family Gadidae is found at such great depths within a broad depth range. Although CLEMENS and WILBY (16) express the belief that this species will be taken only in deep trawling on scientific expeditions, there is a possibility that its range may extend into shallower water than observed.

10. Commercial bottom-fisheries

10.1 *Long-line fisheries*

Only two species of bottom fish, halibut (*Hippoglossus stenolepis*) and sable-fish (*Anoplopoma fimbria*) are taken exclusively by long line. Lingcod (*Ophiodon elongatus*) are also fished as a rule by hook and line in the Strait of Georgia but a small percentage is taken incidentally by trawl. Off the west coast of Vancouver Island and in Queen Charlotte Sound they are exploited almost exclusively by trawls. Generally, halibut is fished to a depth less than 100 fathoms although it exists deeper. Halibut fishing grounds exist fairly near the coast along the northern part of BC and South-east Alaska as well as in the Bering Sea [THOMPSON and VAN CLEVE (17)]. Sable-fish, well known in the smoked-fish trade as "smoked Alaska black cod", is taken mainly off South-east Alaska and the Queen Charlotte Islands at a depth of 150 to 250 fathoms. Long-line vessels do not turn to sable-fish until the halibut season is over, so that catches are light during May and June, with a peak in July or August. While the sable-fish fishery extends for 1,200 miles from Cape Flattery into the Gulf of Alaska, 50% of the BC catch is taken on the northern fishing grounds off Alaska [KETCHEN and FORRESTER (18)]. The habitat of lingcod is near the bottom, ranging from the intertidal zone to at least 60 fathoms, among kelp beds and reefs, especially where there are strong tidal currents [WILBY (19)].

10.2 *Trawl fisheries*

Of the flounder-like fishes taken by trawl, four species are commercially important. The brill (*Eopsetta jordani*) or petrale sole, as it is known to American fishermen, is fished along the open coast at depths from 50 to 250 fathoms. A large stock of this flat-fish was recently found to exist deeper than ever fished before when a winter population was discovered in a small trough of 250 fathoms maximum depth, off the west central coast of Vancouver Island [ALVERSON and CHATWIN (13)]. The lemon sole (*Parophrys vetulus*), commonly known as the 'English' sole in the US, is fished on banks at 20 to 50 fathoms deep in Hecate Strait, Queen Charlotte Sound, and in the Strait of Georgia, where the bottom is composed of soft sand or mud. In some areas, however, it has been taken at depths as great as 110 fathoms. Like most flat-fishes, the lemon sole makes only small seasonal spawning and feeding migrations [KETCHEN (20)]. The rock sole (*Lepidopsetta bilineata*) is a shallow-water species inhabiting hard, sandy bottoms at depths down to 25 fathoms in Queen Charlotte Sound and Hecate Strait. In Alaskan waters, it has been taken from a depth of 70 fathoms (16). Butter soles (*Isopsetta isolepis*) is found on soft, silty bottom in shallow water off the east coast of the Queen Charlotte Islands. It migrates into Skidegate Inlet on the central east coast of these

islands for spawning [HART (21)], where the channel entrance shoals to 10 fathoms and the main inlet is only 30 fathoms deep.

Of the round ground-fish, the gray cod (*Gadus macrocephalus*) is the most important on the BC coast [KETCHEN (22)]. Although fished inshore, primarily by otter trawls, it has been taken off the coast of Alaska and in the Bering Sea by an active line fishery. Gray cod is generally taken in 50 to 70 fathoms deep in winter and is found in shallower water of 20 to 40 fathoms during the summer.

About 22 species of rockfishes have been found in BC waters. The vertical range of the various species extends from tide-water to depths greater than 800 fathoms. The spiny-cheeked rockfish (*Sebastolobus alascanus*) is considered by fishermen as one of the choicest of rockfish and has been taken at a depth of 822 fathoms. *Sebastodes alutus*, known to American fishermen as Pacific Ocean perch, is also a deep-water rockfish which came into high demand in the US after 1950 (13).

Sea-perches (family Embiotocidae) comprise about 20 genera distributed along the shores of the North Pacific. They occur in schools and live as a rule in the shallow waters along rocky or sandy beaches or around wharves and kelp beds. The main concern with these fishes, as well as with the rockfishes, in radioactive waste disposal is that they tend to seek out prominent objects on the sea bottom as a source of food and protection.

11. Status of present knowledge and needed research

Virtually nothing is known about the behaviour in the sea of different types of canisters that might be used for radioactive waste disposal. Research effort should be expended in determining the containment and life expectancy of drums and vaults used at present. If they do not meet required standards, development of suitably engineered canisters should be carried out. The possibility of using hermetically-sealed and well-shielded vaults for radioactive waste disposal in soft muds near shore should be explored.

A large gap in our knowledge exists in the realm of deep-water circulation. Filling this gap is important in order that we might better understand the movement of leached radioactivity from canisters deposited on the bottom. Diffusion characteristics of these deep waters are still largely unknown.

Since bottom sediments play a vital role in adsorbing certain elements by ion exchange, more information should be gathered by research on this phase of radioactivity concentration. The bearing such a process might have on bottom invertebrates and fish should also be investigated.

The effect of radioactivity on fish has been considered largely in its relationship to utilization of the fish by man. Some information is available on the somatic effects of radiation and the levels required to produce morphological damage or death in marine organisms. But virtually nothing is known concerning the genetic effects of radiation on fish. The results of relatively low-level radiation exposure on various stages of fish over long periods of time must be studied in order that genetic damage can be observed.

Experiments have been conducted in a number of laboratories on the uptake, accumulation, and loss of radionuclides in various fishes [BOROUGHS *et al.* (23)]. This type of research must be extended to include many of the commercially-important bottom fishes. The type and quantity of food and feeding habits of many fishes are still unknown. A question which is largely

unanswered at present is the process by which radioelements pass through membranes (uptake) and where and why they concentrate in the organisms. The efficiency of ingestion and digestion of food by organisms and the excretion of elements into the water in various trophic levels must be known in order to evaluate the transport of elements through the different trophic levels.

Areas proposed for radioactive waste disposal should be thoroughly surveyed for the biota and sediments prior to such disposal. During the course of disposal, complete monitoring surveys should be conducted. Some excellent results have been derived from monitoring experiments in the Irish Sea adjacent to the Windscale Works from which radioactive effluent is piped into the sea [DUNSTER and FARMER (24); DUNSTER (25, 26)]. Contamination of the sea-bed, seashore, seaweed, and fish was shown to be low enough during the first few years of operation that it was recommended that discharge be increased from 1,000 curies per month to 10,000 curies per month.

The distribution, migration, and feeding habits of many of the bottom fishes have still to be fully explored. Such information will be very important towards selection of suitable disposal sites, preferably where there are no fisheries.

Ecological studies at typical waste disposal sites should be conducted before and after waste is discharged in order to evaluate the changes which occur—immediate, seasonal, and long-term. The effect of steady-state conditions in continuous radioactive waste disposal should be studied at all trophic levels as well as the exchange of radiomaterials between these levels. A link in the food chain may be greatly changed by the radioactivity and may subsequently affect other trophic levels which have economic significance.

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Primary Productivity and Fertility at Station "P" in the North-East Pacific Ocean

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I. Introduction

The weathership "St. Catharines" of the Canadian Department of Transport patrols at a position 50°N, 145°W in the north-eastern Pacific Ocean (Ocean Station "P") for alternate six weeks periods. There is a sizable and well equipped laboratory built into the ship and full facilities for overside oceanographic operations are available. Regular meteorological observations are made and for the past four years programmes of physical and biological oceanography have been undertaken during each patrol.

Ocean Station "P" lies within the subarctic water mass of the north-east Pacific Ocean (Fig. 1). This subarctic water mass is characterized by its vertical distribution of salinity which consists of three layers; 0–100 m, an almost homogeneous zone of relatively low salinity; 100–200 m, a halocline in which salinities increase by about $1‰$ and which marks the limit of seasonal mixing; and, at greater than 200 m, a deep zone in which salinities gradually increase with depth. Temperature-salinity relationships observed during the present study (Figs. 2 and 3) are typical of the subarctic water mass.

A current enters the area surrounding Station "P" from a westerly direction and divides east of the station to form the south-flowing California Current and the counter-clockwise Alaska Gyral. The centre of the Alaska Gyral, where there is slow upwelling, lies about 300 miles north-west of Station "P". To the south, gradients of salinity and temperature increase to form the Polar Front, between 38° and 46° north latitude.

The location of Ocean Station "P" is truly oceanic, being about 500 miles from the nearest part of the continental shelf. It is reasonable to suppose that, for biotic considerations, the position is typical of much of the eastern subarctic Pacific Ocean.

The work described here was undertaken during a six-week period between 10. July and 21. August 1959, with the purpose of monitoring the summer productivity at a fixed point in the open ocean. To the best of our knowledge

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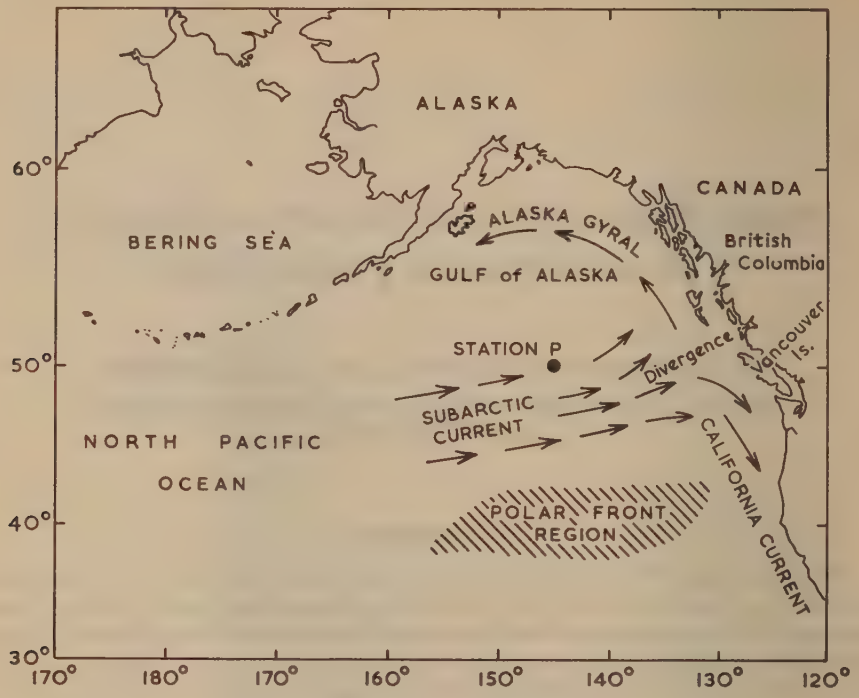

Figure 1. Location of Ocean Station "P".

the primary productivity and fertility of a temperate ocean has not before been continuously measured *in one place* over such a protracted period.

The programme was also the most diverse study of its kind so far reported. The basic programme consisted of the weekly measurement of a wide variety of nutrients, of the standing crop of (microscopic) organic material, and of photosynthetic production in the euphotic zone. The variation of organic matter with depth and location (over traverses of a few miles) was determined and, in order to decide upon the significance of certain observations, a culture experiment was attempted using some 200 l of surface water with its endemic plant cells.

II. Methods

Full working details of the methods used are to be issued in a forthcoming Bulletin of the Fisheries Research Board of Canada. The following notes indicate the general type of approach. A statistical estimate of the precision of each technique has been determined under realistic working conditions.

1. Light

A continuous recording of solar radiation was obtained by an Epply pyrheliometer. The unit used in this paper, the "langley", is equal to one calory of radiant energy per square centimetre of surface. The light attenuation properties of the

sea water were measured from a seven-metre boom at the stern of the ship by matched underwater and deck Weston cells covered by 2 mm of Schott BG 12 glass. The wavelength of peak response of this assembly was about 4300 Å. Vertical extinction coefficients were measured whenever practicable and were generally constant throughout the euphotic zone. The extinction coefficient at 4300 Å was used to characterize the water mass using Jerlov's data (Jerlov, 1951) and hence the total intensity at any chosen depth could be calculated with sufficient precision for biological purposes.

2. Nutrients

Salinity was measured by an electrical conductivity salinometer, *oxygen* by the Winkler method, *pH* using a glass electrode standardized by standard phosphate buffer at pH 6·87, *alkalinity* by the Anderson-Robinson method (Anderson and Robinson, 1946), *silicate* by the method of Mullin and Riley (1955 a), *nitrite* by the method of Bendschneider and Robinson (1952), *nitrate* by a modification of the procedure described by Mullin and Riley (1955 b), and *phosphate-phosphorus* by a modification of the method of Robinson and Thompson (1948). *Soluble organic phosphorus* and *particulate phosphorus* were determined as phosphate after a wet oxidation with perchloric acid (cf. Hansen and Robinson, 1953). The particulate phosphorus is defined as that retained on a Millipore HA filter. The soluble organic phosphorus was measured as the difference between the total phosphate in a sample passed through a Millipore HA filter and the inorganic phosphate in the same sample. *Particulate reactive iron* and *ammonia* were estimated as described by Strickland and Austin (1959). The method used for *manganese* was based on the catalyzed oxidation of the leucobase of malachite green by periodate, as developed by one of the present authors (see also Yuen, 1958).

The levels of iron, manganese, and soluble organic phosphorus in the euphotic zone were estimated from pumped samples (see later). The remainder of the analyses were made on samples taken in 5 l capacity, all plastic, Van Dorn bottles with stainless-steel fittings.

3. Particulate organic material

Sampling and concentration

Weekly samples for the measurement of the amount of particulate matter in sea water were obtained by pumping sea water integrated from a depth of 0 to 50 m. This was accomplished by raising and lowering a hose and intake valve by means of an electrical winch. The hose and pump were constructed of neoprene and the unit had a delivery rate of 20 l per minute. Water was collected in a 200 l polyethylene container and sub-sampled into 8 litre sized polyethylene bottles. Large zooplankters and detritus were removed from the water by first straining it through a nylon net with a mesh size of about 150 microns. The concentration of the particulate matter in the resulting samples was accomplished by filtering the water through a Millipore type AA filter, previously treated with magnesium carbonate (Parsons and Strickland, 1959). When sufficient sea water had been filtered the Millipore filter was removed and the concentrated particulate material was washed from the surface with a small quantity of 3·5% saline. The suspended particulate matter and magnesium carbonate were then further concentrated by centrifugation and the supernatant

liquid was discarded. The resulting residue was used for the determination of carbon, carbohydrate, protein, and fat.

All apparatus involved in the determination of particulate matter was cleaned in acid dichromate or detergent and well rinsed prior to use. There was no evidence of contamination from external sources during the course of these experiments.

Plant pigments

Chlorophyll *a*, *b*, *c*, plant carotenoids and animal carotenoids were measured as described by RICHARDS with THOMPSON (1952) using the Millipore filter technique of concentrating the phytoplankton cells as described by CREITZ and RICHARDS (1955). Extinctions were measured in 10 cm cuvettes and a correction for turbidity was made using the extinction at a wavelength of 7500 Å, multiplied by a predetermined factor to allow for increased light scatter at decreasing wavelengths. Chlorophyll *c* and carotenoids are reported in the somewhat arbitrary units suggested by RICHARDS, the SPU, or better SPPU, specific plant pigment unit.

Carbon

Particulate carbon was determined by wet oxidation with potassium dichromate in concentrated sulphuric acid by a modification of the procedure described by JOHNSON (1949). The decrease in the extinction of dichromate solutions at a wavelength of 4400 Å acted as a measure of the amount of oxidizable carbon.

Protein

Particulate protein was determined using the phenol reagent of FOLIN and COICALTEU (1927). Protein was solubilized by treatment with 1 N sodium hydroxide for 1 hour at 100°C and the colour developed after the addition of the phenol reagent was measured at a wavelength of 7500 Å. The method was standardized on egg-albumin.

Carbohydrate

Particulate carbohydrate was determined by the anthrone reaction using the reagents employed by HEWITT (1958) and measuring the absorption of the colour developed at a wavelength of 6200 Å. Additional absorptions were measured at wavelengths of 5500 Å and 6500 A in order to determine, qualitatively, the presence of hexuronic acids and pentoses. The method was standardized on glucose.

Fat

The amount of fat present in the particulate matter was determined by a modification of the method described by MUKERJEE (1956). The method specifically measures fatty acids and results obtained by the use of this method may be lower than values obtained by an ether extraction technique. The method was standardized on stearic acid.

4. Photosynthetic rate measurements

Rates of photosynthesis were measured using a modification of the technique described by NIELSEN (1952).

Sampling was always done between 0630 and 0700 hours local time (Zone

+ 10) using Van Dorn plastic samplers at depths of 0, 5, 10, 15, 20, 30, and 50 m. Aliquots for measurements were drawn into 300 ml BOD (Biological Oxidation Demand) bottles. (It was found that wire clamps on the stoppers of these bottles were necessary to prevent leakage.)

Aliquots were taken from each depth for measurement both in a shipboard incubator and suspended at their original depths from a free floating buoy. In addition, aliquots of a composite sample, prepared from equal parts of water from each depth, were both illuminated in the incubator and suspended in the sea. The buoy was released from the side of the ship, left in the sea between 0930 and 1530 hrs., a period of relatively uniform illumination, and was tracked by radar, thus freeing the ship for other duties.

Samples measured aboard ship lay horizontally in running sea water and were illuminated from above by blue and green fluorescent lamps in the lid of the incubator. By controlling the voltage, light intensity was kept constant at about 0·08 langlies per minute of photosynthetic radiation. The spectral energy distribution of the illumination approximated to that found at the depth of maximum photosynthesis in oceanic waters.

Samples were filtered through Millipore HA membranes of 0·5 micron pore diameter. It was found that a rinse with 0·002 N hydrochloric acid decreased sample activity by an amount proportional to the volume of acid used and an acid wash was therefore omitted. Sample activities were measured aboard ship within 24 hours of filtration, using a windowless flow counter, and counting rates were corrected for dark uptake and variations in counter sensitivity.

When bad weather prevented buoy releases, the *in situ* production of the ocean was estimated from a knowledge of the rates measured in the ship's incubator, the estimated *in situ* light intensities in the sea and a curve showing the relative rate of photosynthesis versus light intensity. The latter was constructed from the mean of several determinations made during the cruise.

5. Culturing experiment

Water from about 10 m depth was collected in Van Dorn bottles and used to fill a large polyethylene vat holding 185 litres. The vat was fastened to the rail on an upper forward deck, clear of any shadow from superstructure. The water was strained through nylon net of mesh size 70–100 microns to remove all but the smallest zooplankton. No significant quantity of phytoplankton was removed by the net. The top of the tub was covered by two thicknesses of 0·1 mm polyethylene sheet and the sides surrounded by sacking. Surface sea water was played on to the sides of the vat from a hose, so that the mean temperature of the culture was only about 2–3°C greater than that of the upper euphotic zone. The level of illumination in the vat, as measured by a photocell, was about 20% of the incident daylight, corresponding to the light level at about 20 m depth in the sea.

The contents of the tub were stirred three times a day and samples taken for analysis about noon. The carbon uptake was measured by sampling the water into a light and dark BOD bottle first thing in the morning, inoculating these bottles with radioactive carbon, and suspending them in the centre of the tub all day. In the evening the carbon uptake in both bottles was measured and equated to the increase of plant carbon. Allowance was made for night-time respiration by assuming that its hourly rate was about one-tenth of the mean hourly rate of photosynthesis. The results were recorded as daily means at each

noon, a day being taken as the period from midnight to midnight. In view of the nature of the measurement and the various assumptions made, the estimated uptake of carbon is probably low rather than high.

On alternate days the photosynthetic potential of the water was measured (using radioactive carbon) by illuminating a BOD bottle of water in a constant light incubator, to give a value for hourly photosynthesis that was independent of variable sunlight conditions. The content of the tub was analysed periodically for silicate, nitrate, and phosphate and the particulate material was analysed for carbon, carbohydrate, protein, and lipid. The pigment content was measured daily.

III. Results

A complete tabulation of all the results obtained on this cruise has been published as a Data Record by the Fisheries Research Board of Canada (McAllister, *et al.*, 1959). A limited supply of these reports are available and can be obtained from the Pacific Oceanographic Group, Nanaimo, B.C., Canada. In the present paper we are presenting only those data which seem to us to be of major importance or which serve to illustrate general trends and magnitudes.

1. Light

The vertical extinction coefficient (metre^{-1}, base 10 logarithms) at 4300 Å was roughly constant in the first 50 m and had a value of about 0·035 for much of the cruise. At some time in the second or third week of August the water cleared substantially, the vertical extinction coefficient decreasing to about 0·025.

2. Nutrients

A typical set of nutrient profiles is shown by Table 1, the data being obtained about halfway through the cruise. Such analyses were carried out once a week, giving seven profiles in all, and were restricted to the surface waters down to

Table 1

Nutrient profiles Station N 3 (24. July 1959)

Depth m	Temp.*) °C	S*) ‰	O_2 mg at/l	pH (*in situ*)	Total alk.	SiO_3 µg at/l	Nitrogen µg at/l			PO_4 µg at/l	N/P
							NO_3	NO_2	NH_3		
0	11·2	33·12	0·585	8·20	–	23·7	9·5	0·09	0·3	1·24	7·7
10	11·2	32·78	0·588	8·22	2·31	24·4	9·4	0·11	–	1·29	7·3
20	11·2	32·79	0·584	–	–	23·5	9·5	0·12	–	1·26	7·5
29	10·8	32·76	0·586	8·23	–	21·7	10·5	0·15	0·4	1·40	7·5
48	7·3	32·87	0·627	–	–	32·4	14·1	0·17	–	1·69	8·3
73	4·6	32·98	0·634	–	–	34·9	16·4	0·94	<0·2	1·83	9·0
97	4·5	32·98	0·632	8·02	–	32·9	17·3	0·64	–	1·87	9·2
121	4·7	33·55	0·500	–	–	48·7	25·6	<0.01	–	2·27	11·3
145	4·9	33·83	0·380	–	–	57·8	26·4	<0·01	–	2·37	11·1
280	3·8	33·94	0·190	7·78	–	76·9	33·0	0·01	<0·2	2·83	11·7
950	2·8	34·4	0·060	7·67	2·40	121	37·8	0·05	0·6	3·23	11·7

Specific alkalinity 0·124 at 10 m, 0·123 at 950 m.

*) Values measured about one hour earlier on separate cast. Approximate data for reference.

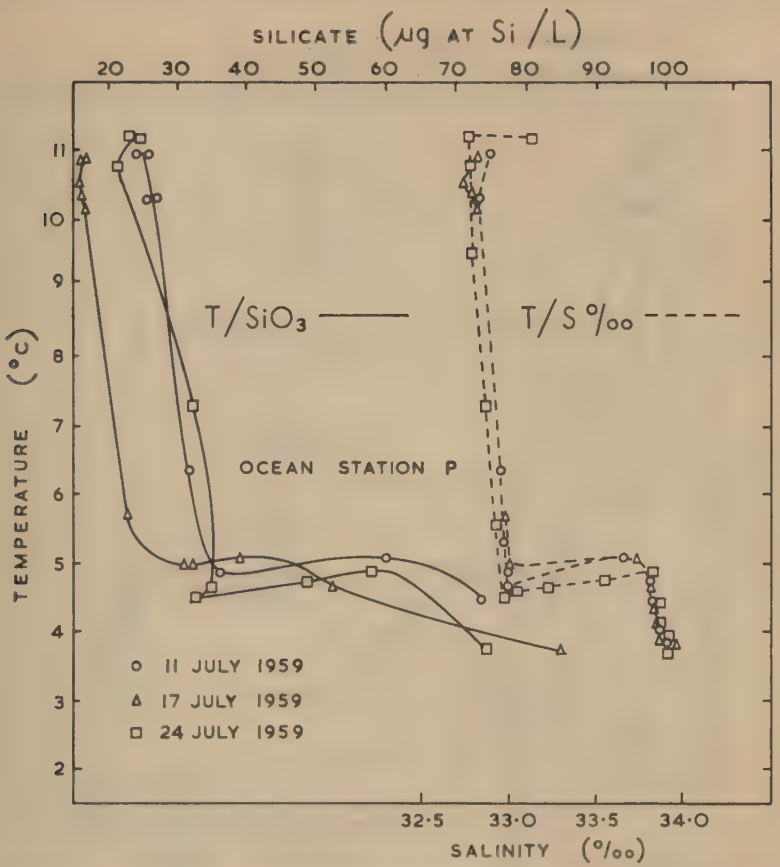

Figure 2. Comparison of temperature-silicate and temperature-salinity curves.

300 m, with a sample from near to 1000 m to establish the concentration levels in deeper layers. The figures for temperature and salinity were obtained from a cast of Nansen bottles "fired" about one hour earlier.

The oxygen content of the top 30 m was in the range 0·57 to 0·59 mg at/l for the entire cruise and pH values (corrected for *in situ* temperature) were between 8·16 and 8·22. The specific alkalinity at all depths was constant at 0·124, within the precision of the method used. Nitrite nitrogen was always present in the top 50 m to about the same extent (0·1–0·15 μg at/l) and always showed a peak around 75 m, where the concentration could be as high as 1 μg at/l. Ammonia concentrations were variable and had no obvious pattern.

Particulate reactive iron (0·03–0·13 μg at/l) and manganese (0·005–0·017 μg at/l) were present in appreciable concentrations in pumped samples. The soluble organic phosphorus ranged between 30 and 65 percent of the inorganic phosphate found in the same water which was of the same order as that found previously by one of us in north-eastern Pacific coastal and oceanic samples (Strickland and Austin, 1960).

The most marked feature of the nutrient picture was the variation, in the

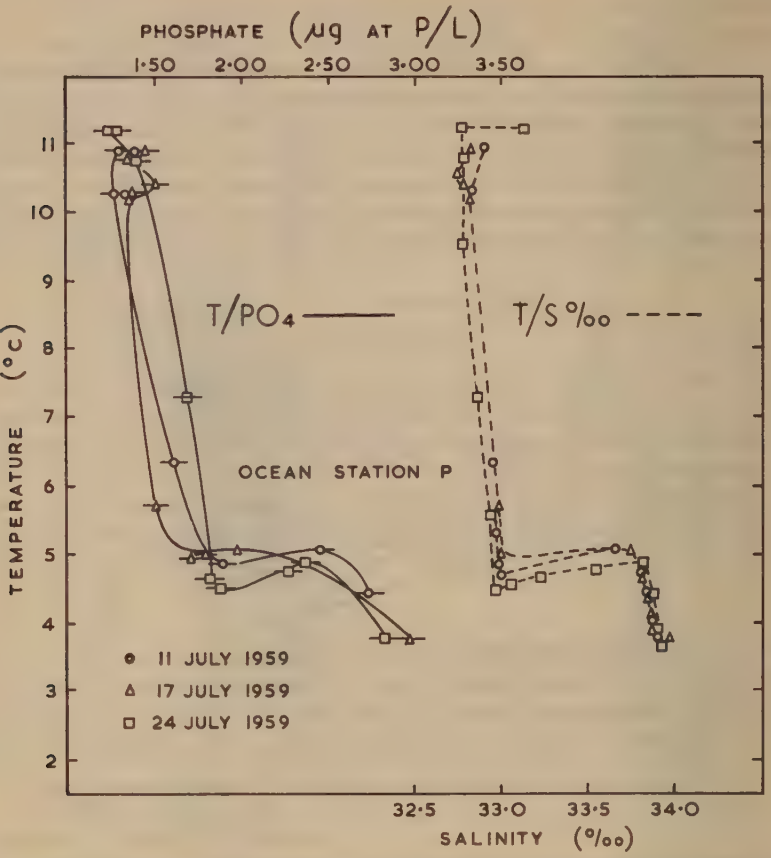

Figure 3. Comparison of temperature-phosphate and temperature-salinity curves.

top 50 m, of the silicate and nitrate concentrations. A similar variation in phosphate concentration may have occurred but was much less. We have illustrated this in Figure 2 where the silicate and salinity are plotted against temperature for three successive weeks. A similar plot for phosphorus and salinity is given in Figure 3. The size of the circles, squares, or triangles in Figure 2 represents, approximately, the uncertainty of the silicate analysis ($\pm 2\sigma$). In Figure 3, a horizontal line shows the $\pm 2\sigma$ uncertainty limits of the phosphate results. At 25 m, about the middle of the euphotic zone, the silicate varied from week to week between 16 and 31 μg at/l, with no obvious pattern. The corresponding range for nitrate was 6 to 12·5 μg at/l. For phosphate, however, the variation was only between 1·23 and 1·33 μg at P/l.

The nutrient levels also changed on a single day as the ship steamed over a traverse of a few miles. In an east-to-west journey of eight miles the silicate at 20 m changed from 26·7 to 22·6 μg at/l. An even more striking change is seen in Table 3 with a north-to-south traverse. These variations are greater than ever noted between surface and 30 m values at a single fixed point and are well outside the range of any possible experimental error.

Table 2

Routine crop station

Plant carbon = 60 × chlorophyll *a* concentration. Crop in mg/m³ or m SPPU/m³ (chlorophyll *c* and carotenoids).
Plant carbohydrate = 47 × chlorophyll *a* concentration.
Plant protein = 35 × chlorophyll *a* concentration.

Station	Date (1959)	Carbon			Carbohydrate			Protein			Lipid	Chlorophylls		Carotenoids
		Total	Plant	Rem.	Total	Plant	Rem.	Total	Plant	Rem.	(Total)	*a*	*c*	
C 1	13. July	240	35	205	100	26	74	125	20	105	13	0·55	0·8	0·52
C 3	20. July	145	20	125	38	14	24	165	10	155	31	0·30	0·5	0·17
C 5	27. July	145	25	120	58	21	37	115	15	100	20	0·44	0·9	0·16
C 6	4. Aug.	130	15	115	72	13	59	150	10	140	22	0·27	0·4	0·08
C 9	10. Aug.	160	35	125	49	29	20	105	22	83	13	0·62	0·8	0·40
C12	17. Aug.	145	15	130	59	13	46	109	10	99	7	0·27	0·4	0·11

Table 3

Stations on traverse; samples from 5–10 m

Nutrients in μg at/l. Crop in mg/m³ or m SPPU/m³ (chlorophyll *c* and carotenoids)

Station C 11	Time GMT	Miles	Position	$HSiO_3$	Inorganic P	Carbon	Chlorophyll *a*	Chlorophyll *c*	Carotenoids
13. Aug. 1959....	1840	0	50°15′N, 145°00′W	16·6 ±0·3	1·35 ±0·08	214 ±30	0·85	1·2	0·7
North-south	2000	10	50°05′N, 145°00′W	20·9 ±0·3	1·30 ±0·08	129 ±25	0·25	0·8	0·1
							(±abt. 0·06)	(±abt. 0·1)	(±abt. 0·1)
Traverse.....	2140	20	49°55′N, 145°00′W	21·0 ±0·3	1·58 ±0·08	120 ±25	0·30	0·2	0·2
At Station "P" ..	2245	30	49°45′N, 145°00′W	17·7 ±0·3	1·28 ±0·08	224 ±30	0·76	1·1	0·5

3. Particulate organic material

The analysis of six composite phytoplankton crops, measured over a period of six weeks, is shown in Table 2. The amounts of carbon, protein, and carbohydrate that could be attributed to phytoplankton have been calculated from the amounts of chlorophyll *a* present, using conversion factors obtained from the culturing experiment (see later). These quantities have then been subtracted from the corresponding metabolite found by analysis and the difference is reported as "remainder" (Rem.), which is an approximate measure of detritus.

The detritus was characterized by a high protein to carbohydrate ratio. However, not all organic material is accounted for in terms of these two metabolites, as may be deduced from the disagreement between total carbon and the sum of the carbohydrate and protein carbon. The amount of fat is low and does not contribute significantly. The cause of the discrepancy may be in the method used for estimating total protein which took into account only the tyrosine and tryptophane contents of a hydrolysate. A more satisfactory procedure has since been developed.

Microscopic examination of the detritus showed that a considerable portion was composed of fibrous material of irregular dimensions which had the appearance and gave a positive stain for cellulose. With the exception of station C1 the amount of detritus was fairly constant at about 125 mg carbon/m^3 in the euphotic zone and was still present in about half this quantity at 1000 m, which was the deepest sample analysed.

Microscopic examination of settled sea-water samples showed that the predominant plant cells were coccolithophores. Further evidence that the crop was composed predominantly of ultra-plankters was obtained from the fact that 75% of the pigment in a water sample passed through a 10-micron filter. Net-phytoplankton (greater than 50 microns) accounted for only a very small fraction of the plant crop. The predominant species of net-phytoplankton were the dinoflagellates, *Ceratium fusus* and *Ceratium tripos* and the diatom, *Coscinodiscus marginatus*.

Maximum pigment concentrations were found at between 50 and 75 m while no pigment at all was detected below 200 m. Particulate phosphorus was less than 1 mg P/m^3 in all samples.

Table 3 shows the variation of phytoplankton crop and particulate carbon over a north-to-south traverse of 30 miles, together with variations in the silicate and inorganic phosphate concentrations over the same distance. It may be seen that at positions 0 and 30 miles the plant pigments are higher than at the two centre stations. At the same time there is a statistically significant difference in the amount of organic carbon at the stations at the beginning and end of the traverse, compared with those in the middle.

4. Photosynthetic rate measurements

Table 4 gives the full results of a typical productivity experiment.

Six determinations of productivity were carried out, at weekly intervals. Table 5 gives the production beneath a square metre per day in the upper 50 metres for each weekly station, together with the total incident solar radiation on the day concerned. The rate of photosynthesis per milligram of chlorophyll *a*, as measured in the ship's incubator (at about 0·08 langlies/min. of photosynthetic illumination) is shown in the fifth column. This relative rate

Table 4

Results from a typical photosynthetic rate determination (Station P 2)

Depth m	Incubator rates (mg C/m³/hr)	Mean *in situ* light (langlies/min. 3800–7200Å)	Rates measured on buoy: *In situ* mg C/m³/hr.	Rates measured on buoy: Composite sample from euphotic zone mg C/m³/hr.
0	1·35	0·45	0·82	0·29
5	0·64	0·19	0·48	0·37
10	0·55	0·11	0·32	0·51
15	0·74	0·07	0·46	0·47
20	0·70	0·04	0·29	0·46
30	0·57	0·01	–	0·29
50	0·51	0·005	0·22	0·05

Table 5

Collected productivity data

Station	Date (1959)	Approx. euphotic zone production (mg C/m²/day)	Total light (langlies)	Rate per unit of chlorophyll *a* (mg C/hr./mg)	Rate per unit of total pigments (mg C/hr./mg)*)
P-1	15. July	390	330	1·34	0·45
P-2	22. July	160	500	1·31	0·47
P-3	29. July	260	280	1·50	0·56
P-4	6. Aug.	310	270	1·83	0·51
P-5	13. Aug.	55	260	1·07	0·23
P-6	19. Aug.	55	260	0·63	0·24
Culture (at 7·5 days)		–	–	1·8	0·6
Diatoms and dinoflagellates held by 40-micron net		–	–	1·0	–

*) Pigment units for chlorophylls *a*, *c*, and plant carotenoids all added together to give a somewhat arbitrary mixed unit.

Mean daily production 205 mg C/m² for the entire cruise.

has also been calculated on the somewhat arbitrary basis of total pigment units (chlorophylls *a*, plus *c*, plus carotenoids). The pigments and rates used to compute these ratios were measured on composite samples taken from the whole euphotic zone.

Weather prevented buoy releases on two days (Stations P3 and P5) and the *in situ* production was then calculated from incubator results. The ratio of the *observed* to calculated euphotic zone production in the other four experiments ranged from 0·8 to 1·2 and averaged at 0·98. A vertical profile of production, measured using the buoy, is shown in Figure 4, with the corresponding curve calculated from incubator results.

It will be noted in Table 5 that although the total photosynthetic production beneath a square metre per day varied over six-fold, the corresponding rates per unit pigment concentration in an incubator varied by little more than two-fold.

5. Culturing experiment

The results of this work are summarized graphically. In Figure 5 the daily value for the plant carbon in the vat is plotted on a log-scale against the duration of the experiment. The initial value of plant carbon has been estimated

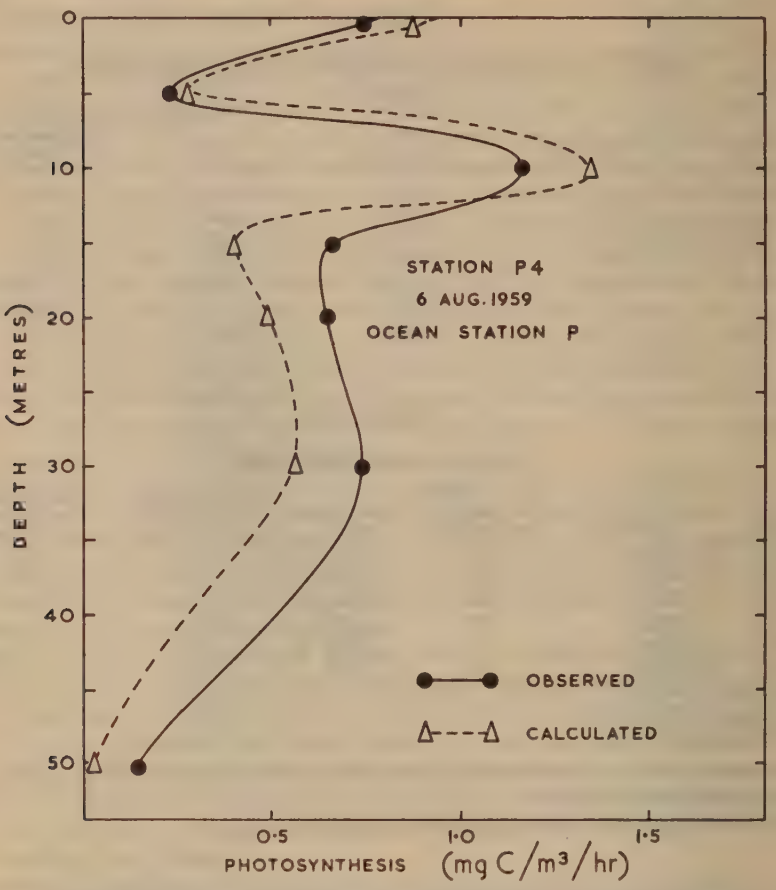

Figure 4. Typical productivity curve, observed and calculated values. Station P. 4. Sixth August 1959.

from the initial chlorophyll *a* concentration in the vat. The correct value could have been in the range 10 to 30 mg C/m^3 but we consider 20 mg C/m^3 the most likely figure. In Figure 6 the corresponding plot is given for the daily plant pigment data. In the top of both these figures there are histograms of the total *daily* radiation in langlies (cals/cm^2 of solar radiation between about 3000 Å and 50,000 Å) shown on a linear scale.

In Figure 7 A the variation of reactive silicon in the tub is plotted against time. The corresponding plots for phosphorus and nitrate (all as mg/m^3 of the element) are shown in Figure 7, B and C. The fourth section of Figure 7 (lettered D) shows the measured values of carbon, carbohydrate, protein, and lipid. The values for carbon were obtained from the oxidizable carbon results after correcting for the amount of lipid measured in the same sample. Analyses are only reported from the seventh day onwards as, initially, the detritus present masked the presence of living plant material. After a few days the detrital matter appeared to separate out by some form of clumping mechanism and could be separated by a fine nylon net. It was largely absent from the samples analysed after about five days.

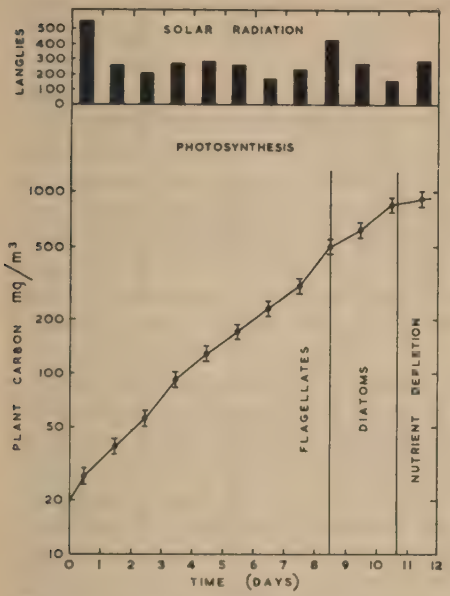

Figure 5. Production of photosynthetic carbon in the culturing experiment.

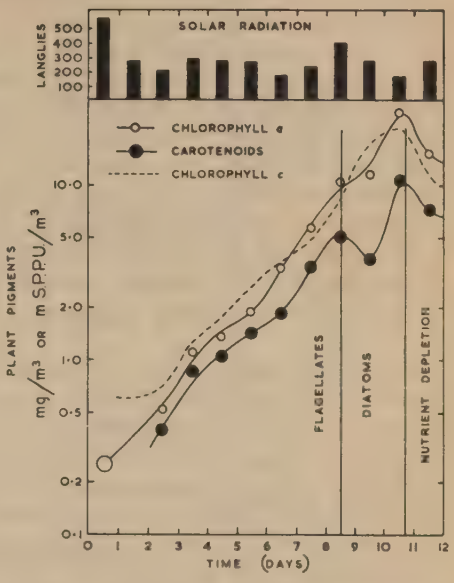

Figure 6. Production of plant pigments in the culturing experiment.

The predominant species in the culture for the first six to eight days was a small flagellate (tentatively *Pontosphaera huxleyi*). A consideration of elementary kinetics shows that this species must have comprised a substantial fraction of the initial plant crop. Between the seventh and ninth day the coccolithophore was rapidly replaced by a small pennate diatom and by the end of the eleventh day its growth was being severely limited by nutrient depletion. These major stages in the progress of the culture are shown in Figures 5, 6, and 7 by vertical lines.

IV. Discussion

It is clear that the level of concentration of the major plant nutrients at Station "P" is adequate for vigorous plant growth throughout the period studied. Occasional analyses, made during the past few years, have given no indication that the situation is ever otherwise. The presence of excess nutrient is confirmed by our culture experiment. Figures 5 and 7 show that plant growth is not seriously impeded by nutrient deficiency until after ten to eleven days of growth, when the nitrate concentration is reduced to less than 1 μg at/l. Phosphate is then still present at an appreciable concentration, about 0·35 μg at/l, as would be anticipated from the initial ratio of nitrogen to phosphorus (9·2:1 on an atomic basis). The N:P ratio in the euphotic zone was always found to be low during this patrol (between about 5 and 9) and even in the deeper waters it never reached 15:1 (see Table 1). If soluble organic phosphorus is included the ratios would be even smaller. When phytoplankters, containing combined nitrogen and phosphorus in a ratio of about 15:1, grow in such water nitrogen must be the first major nutrient to be used up (e.g., COOPER, 1937, 1938).

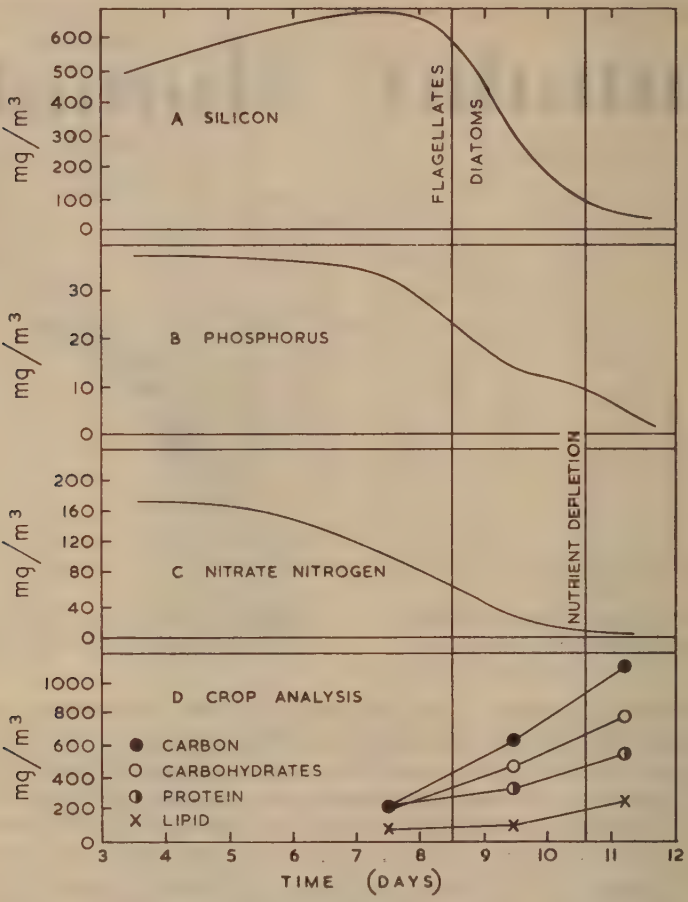

Figure 7. Depletion of silicon, phosphorus, and nitrogen and the formation of metabolites in the culturing experiment.

However, it must be stressed that a ratio of 15:1 for nitrogen to phosphorus in plants is a very rough average and will depend upon the availability of the two elements. Plant cells can adjust their levels of combined nitrogen and phosphorus over quite a wide range. Thus in our culturing experiment the average ratio of N to P abstracted from the sea water by the growing phytoplankters was 11:1 but the ratio was as high as 14:1 during the initial phase of growth, when nitrate was abundant, and as low as 8·5:1 during the final period of nitrogen deficiency, whilst the remaining phosphate was being used up. The complete removal of phosphate from sea waters where the N:P ratio is low can be explained on the basis of plant behaviour and one does not have to suppose a rapid regeneration of nitrate or the addition of atmospheric nitrate to explain the eventual loss of phosphate (cf. NIELSEN and JENSEN, 1957).

It is interesting to note the variability found week after week in nutrient-depth profiles, especially with silicate and nitrate. This is illustrated by Figure 2 in the case of silicate. Nitrate changes were similar but the variations of phosphate

were much less and, although probably significant, could scarcely be differentiated from analytical errors (Fig. 3). *In situ* plant growth cannot be responsible for the variations. At the observed rate of photosynthesis at Station "P" a month or more of phytoplankton growth, with no simultaneous remineralization, would be required to give the changes recorded from one week to another. The variation must be the result of the lateral changes of concentration that are encountered over a distance of only a few miles (see Table 3). The exact position of the ship could easily have differed by a mile or two from week to week. There would have been very little indication of different water masses from T/S curves or from oxygen and phosphate profiles, the three most commonly determined parameters on oceanographic stations, which draws attention to the desirability of using the silicate or nitrate content of surface waters to indicate their biotic origin.

The cause of the variations found near Station "P" is presumably in the past biotic history of two or more water-bodies situated near the ship. T/S variations were too small for these water-bodies to be considered as separate "water masses" in the currently accepted oceanographic sense and it seems necessary to use some such term as "biotic submass" to convey the necessary distinction. Such drastic changes in nutrient profiles over a few miles of sea, which we suspect characterizes much of the region and which cannot possibly have had a *recent* biotic cause, underline the dangers of applying the techniques of Riley (e.g. Riley, 1951) or Steele (Steele, 1956) for calculating primary production from nutrient and temperature profiles. In the vicinity of Ocean Weather Station "P" any results obtained by such an approach would have been completely misleading.

The amount of particulate organic material in the open ocean at 50°N, 145°W was unexpectedly large. The method of sampling removed all but a negligible quantity of whole zooplankters. The remaining organic matter, equivalent to some 100–200 mg C/m^3, was an appreciable fraction of the amount found for much of the year in Canadian coastal waters (unpublished results).

Nearly all the oceanic particulate matter was detrital (between 80 and 90%) and contained much protein, some carbohydrate and rather less lipid. A spot analysis showed very little chitin (about 2 mg/m^3). There were a considerable number of relatively long cellulose fibres present which could have accounted for much of the carbohydrate found in samples (see Table 2) and which were almost certainly of terrestrial origin. The total tonnage of disintegrated wood present in the Gulf of Alaska, if the amount of cellulose fibre at 50°N, 145°W is at all representative, would be truly enormous but it must be remembered that there are many thousands of miles of coastline surrounding the Gulf and the beaches are littered with dead wood to a degree which must be seen to be appreciated. The presence and stability of wood in ocean waters has been remarked upon by several authors (e.g. Atkins, *et al.*, 1954; Bruun, 1957; Barber, *et al.*, 1959). A considerable amount of driftwood and kelp passed the ship during our patrol.

The detrital matter at Station "P"persisted to at least 1000 m and therefore constituted the bulk of potential food-stuff in this part of the ocean. It is unfortunate that we know so little about the availability of such particulate matter as a food for secondary producers.

The origin of the detritus (other than the cellulose) is open to some speculation. From its high protein content and appearance under a microscope one

may assume it to be largely of animal origin and some fragments of zooplankton could be recognized. Whether or not this detritus is endemic is not known, as we have no knowledge of the long-term stability of such material. Ocean currents in the top few hundred metres are such that coastal water could eventually reach the location of Station "P" from Asia or from the Canadian and Alaskan coasts but time factors are not known with any certainty.

In a north-to-south traverse (data shown in Table 3) the concentration of both detritus and plant cells change, both decreasing in the centre portion of the traverse where the water had a higher silicate content. This further underlines the presence of different water-bodies within a few miles of the ship's nominal position but the simultaneous decrease of both detritus and plants may have been fortuitous. In an east-to-west journey there was no such obvious relationship.

The maximum concentration of plant material, as indicated by pigments, occurred at around 75 m, at the very top of the halocline, and it is interesting to see that nitrite concentrations always had a marked maximum in the same region (cf. BRANDHORST, 1959), irrespective of the biotic submass of water as characterized by other nutrient profiles. It would appear as if nitrification processes are most active at a depth where there is an accumulation of relatively recent plant detritus; certainly the nitrite disappeared at depths where plant pigments were also absent.

The summer rate of primary productivity at Ocean Station "P", which in round numbers was 7 mg C/m^3/day near the surface or some 200 mg C/m^2/day for the entire euphotic zone, was of the order to be anticipated from the few other observations that have been made in the North Pacific. Values in the Pacific range from 1 mg C/m^3/day near the equator and in an area south of the Polar Front to some 50 mg C/m^3/day at a fertile area by the Aleutian Islands. The more productive parts of the subtropics and much of the northern ocean appear to have a productivity similar to that found during the present investigation (e.g. KING, *et al.*, 1957; NIELSEN and JENSEN, 1957; HOLMES, 1958).

Throughout our patrol the primary production was almost entirely from ultra-plankton that passed through the finest netting (cf. WOOD and DAVIS, 1956; NIELSEN and JENSEN, 1957) or even through a sintered-glass disc that filtered out cells larger than 10 microns. This provides yet further evidence, if further evidence were required, of the impracticability of plankton nets for quantitative work in oceanic waters, even in northern latitudes.

The productivity beneath unit area varied six-fold throughout the patrol but only about three-fold for most of the time. The variation was due mainly to differences in the concentration of plant cells in the euphotic zone and to differences in the distribution of these cells with depths. As with many other factors, major changes could best be attributed to the ship passing through various water bodies rather than to any great change within the same water mass. This is borne out by the observation that the rate of photosynthesis per unit concentration of chlorophyll *a* (at a standard light intensity) was fairly constant throughout the patrol (Table 5), especially when allowance is made for experimental errors in estimating chlorophyll. If the rate of photosynthesis were related to the sum of *all* plant pigments in an arbitrary mixed unit (CURRIE, 1958) the ratio was even less variable (Table 5).

In the culturing experiment the corresponding rate of photosynthesis per unit of pigment was a little higher than that found in nature. However this

difference is probably due to the population in the culture adjusting to a more uniform light intensity and a slightly higher temperature than that found in the euphotic zone.

After some seven to eight days the population in the polyethylene vat changed radically and a diatom began to predominate (this is shown very strikingly by the silicate curve in Figure 7 A) but for the first four to six days of growth the cells present were almost certainly major constituents of the initial population. There was no evidence of any sudden change of growth-rate during the initial period of culturing due to a "wall effect" and, in fact, there was a remarkably good approximation to steady exponential growth throughout the entire experiment if we allow for minor effects of solar radiation (Figure 5).

The amount of carbon in the culture doubled itself about once every 1·75 days although, as mentioned above, this rate would probably have been a little slower under more natural conditions. The doubling time found in nature, for the crop beneath a unit area of sea surface, was some four to five days (cf. the value of 4 days estimated by Nielsen and Jensen, 1957). This decrease of growth-rate is to be expected as the average illumination within the euphotic zone was much lower than that received by the culture. Even with a doubling rate of four to five days, however, the maximum variation of standing plant crop found throughout the entire six-week patrol could have occurred in one week of uninterrupted growth.

We thus have a situation of potentially "explosive" growth held in check by some mechanism that almost exactly balances the photosynthesis. Considering that much of the observed change of crop concentration during the patrol arose from the presence of different water-bodies, the constancy of standing crop is quite remarkable and "spot" analyses made before and since this patrol have indicated that the level throughout the year, even in December, is similar to that found in July and August. There is certainly no evidence of a phytoplankton "bloom"of the magnitude found in the culturing experiment which, in turn, was reminicent of the growth pattern observed in British Columbia coastal waters, where the nutrient and light levels are comparable to those at Station "P" and where the rate of photosynthesis per unit of plant pigment is also similar (cf. Currie, 1958).

If the population level is being held steady solely because plant cells are sinking from out of a 50–70 m euphotic zone, then a sinking rate of well over 10 m a day is required. No really satisfactory sinking rates have yet been determined in nature but a value of 10 m a day is at the upper limit of any suggested values and then only for tropical seas or for large phytoplankters (Allen, 1939; Riley, *et al.*, 1949; Steele, 1956; Nielsen with Andersen, 1957). A sinking rate of 10 m a day is most improbable for ultraplankters in cold northern waters (some of which we observed, experimentally to be positively phototactic) and it may be assumed with some confidence that sinking can only explain a small fraction of the inferred population loss.

The only other loss mechanism (ignoring natural mortality) is by grazing. This conclusion, namely that a low and relatively constant oceanic crop is the result of grazing, is not new and has been suggested by several workers (e. g. Nielsen and Jensen, 1957; Currie, 1958; Cushing, 1959). Most previous studies and theoretical treatments have been concerned with tropical oceans (in particular the Sargasso) where the nutrient levels are low and it has generally been assumed that some form of nutrient deficiency acts as the final control

preventing large population increases. Grazing has been thought of as the mechanism for levelling growth peaks and regenerating plant nutrients.

However, from the present work at Station "P" it appears that *grazing alone* must be largely responsible for keeping the population in check against a natural tendency to grow to some fifty times its observed concentration. Such a control is quite remarkable, even assuming a fairly constant population of herbivorous zooplankters with a lengthy reproductive cycle, and there is no obvious reasons why the standing crop of phytoplankton (and hence the whole biotic level) could not have been several times greater than was found.

Unless conditions at 50°N, 145°W are abnormal, and we have no reason to suppose this, it appears as if the low concentration of standing crop in this northern ocean is the result of constant overgrazing, whereas near the adjacent coasts, which have water of essentially the same fertility and illumination, the plant crop can increase to the maximum possible level by a "classical" cycle of blooming and grazing. If the explanation of this difference lies in the ecology of the grazing populations, the only obvious difference between the two environments lies in the water depth. In relatively shallow coastal areas creatures at higher biotic levels are dispersed in a depth of water which is comparable with the depth of the euphotic or the mixing layers. In the open ocean a complex vertical feeding pattern exists throughout a column which may be fifty or more times as deep as the euphotic layer.

Acknowledgements

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Summary

1. For a six-week period in July–August 1959, weekly measurements were made at Ocean Station "P" (50°N, 145°W) of all major nutrients, the standing crop of particulate organic matter and the *in situ* rate of phytoplankton photosynthesis. Two-hundred litres of water from the euphotic zone were freed from zooplankton and the endemic plant cells cultured under conditions comparable with those found at 10–20 m depth in the sea.

2. Particulate organic matter was present in surprisingly large amounts for an ocean area, equivalent to some 100–200 mg C/m^3 in the surface waters, and consisted mainly of detritus, persisting to a depth of many hundred of metres. The material was largely proteinaceous but about 40 mg/m^3 of carbohydrate (as glucose) were present, some of which is thought to be derived from disintegrated wood fibres of terrestrial origin. There was very little lipid (about 15 mg/m^3 as stearic acid) and only a trace (2 mg/m^3) of chitin.

3. Certain aspects of the nutrient analyses are discussed, in particular the N:P ratio. A distinct "patchiness" was found in the distribution of silicate, nitrate, and particulate matter in the euphotic zone over a lateral traverse of only a few miles. This presumably arises from the presence of "submasses" or fronts of waters of different biotic origin. The changes could not have resulted

from *in situ* productivity at the time of the patrol and they were not shown up very well by the more commonly measured profiles of phosphate, oxygen, or salinity.

4. The standing crop of phytoplankton was relatively constant throughout the six weeks (15–35 mg C/m^3) and the net photosynthetic productivity averaged at some 200 mg C/m^2/day, as measured by the radioactive carbon technique. Practically all the productivity was from organisms passing the finest plankton netting or even a coarse, sintered glass filter. Fair agreement was found between *in situ* productivity, measured in bottles suspended from a free-floating buoy, and the rates calculated from "incubator" results at a constant light intensity and a knowledge of the attenuation of light in the euphotic zone.

5. Neither nutrients, temperature, nor light were limiting growth factors. Left to itself the endemic crop from the ocean could bloom to fifty times its observed level before growth was arrested by nitrate depletion. The tight control of this "explosive" situation found in nature is considered to be the result of grazing and sinking, mainly the former. It is remarkable that such a stable condition is possible and that overgrazing should persist to such inefficient extremes whilst there is a large excess of nutrients still present.

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Commercial Landings of Atlantic Salmon 1910 THROUGH 1959

Long-term fluctuations

During 1910 to 1959, the average annual commercial catch of salmon was 6.8 million pounds.

Commercial landings around 1910 and 1920 were almost as low as in recent years. There also were low catches around 1880, 1890, and 1900.

In the 1959 season, commercial nets took 4,130,000 pounds, or 61% of the long-term average.

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POPULAR interest in comparing recent salmon catches with those of the "good old days" indicates the value of a chart to show total commercial landings in eastern Canada over a long period.

Figures for commercial catches of Atlantic salmon in Newfoundland, the Maritime Provinces and Quebec are available back to 1870; however, the accompanying chart covers only the years from 1910 to 1959. For that period the statistics were calculated on the same basis and can be assumed to maintain the same degree of accuracy.

THE Newfoundland figures were compiled by the St. John's office of the Federal Department of Fisheries' Markets and Economics Service, and include estimated local consumption of salmon caught by commercial gear. Data for the other provinces were obtained from reports of the Dominion Bureau of Statistics, supplemented for recent years by reports of the Halifax office of the Department of Fisheries and the Quebec Bureau of Statistics.

The chart should be a useful addition to commercial and angling catch statistics obtained since 1949 and reviewed in reports on the Atlantic salmon research and management program that have been published annually since 1955 in the Department of Fisheries' "Trade News". Reliable statistics on salmon angling catches before 1949 are available for only limited stretches of a few rivers. It is impossible, therefore, to review long-term variations in the success of the sport fishery for the whole Atlantic area, as can be done for the commercial fishery.

THE upper solid line of the chart joins points that represent the total weight of salmon landed each year in eastern Canada. The average annual total catch of the 50-year period is shown by the horizontal dotted line at 6.8 million pounds. The lower part of the chart gives the annual landings in each province, which together make up the totals shown by the upper line.

Above-average total catches occurred mainly from 1921 to 1942. The outstanding peak occurred in 1930 because of an exceptional catch in Newfoundland. In New Brunswick, Quebec and Nova Scotia there were peaks in the 1924-1926 period similar in size to that of 1930.

MANY people can recall excellent salmon fishing around 1930 and sometimes assume that such was the case as far back as the start of the fishery. The chart shows that around 1910 and 1920 the landings were almost as low as in recent years. Still earlier than the period covered by the chart there were similarly low catches around 1880, 1890 and 1900, with intervening peak years of production.

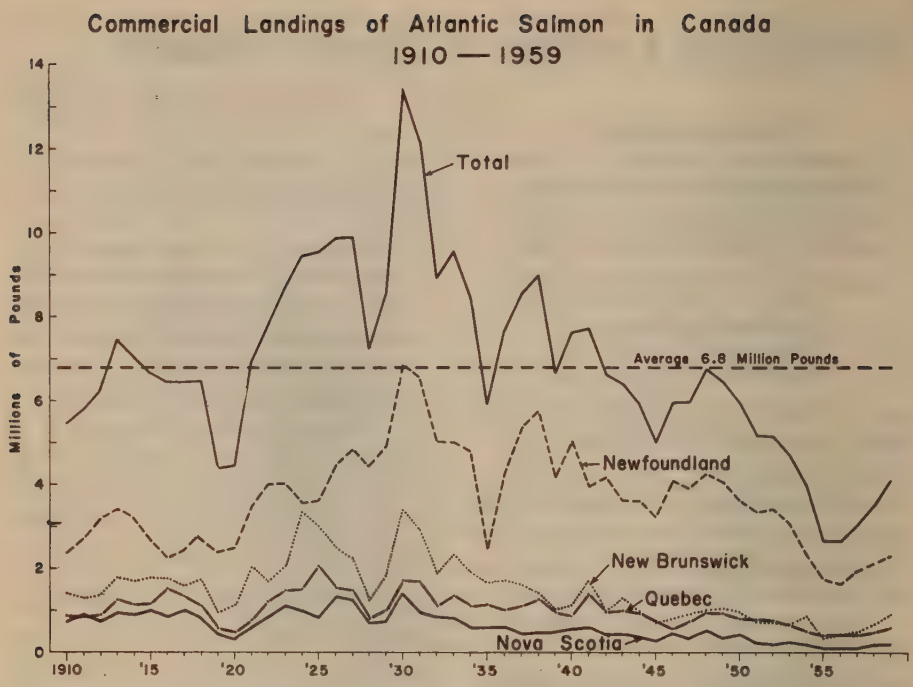
In 1955 and 1956 commercial landings reached the lowest level of the past 50 years, but there has been steady improvement up to the end of the 1959 season. In 1959 the total catch, at 4,130,000 pounds, was 61% of the long-term average. It is desirable to compare recent production with the average annual production in this way, rather than with exceptional catches in peak years.

THE January 1955 issue of the *Atlantic Salmon Journal* had on page 28 a chart showing salmon catches of the 1906-1945 period in the British Isles compared with catches in our Maritime Provinces. It may be of interest to compare it with the present chart. As would be expected the addition of Newfoundland and Quebec figures to the Maritime statistics to give a Canadian total gives a similar picture of fluctuations about a long-term average.

Both in Canada and Great Britain there were, for example, high catches about 30 years ago followed by a severe falling off in production. As pointed out in 1955, this suggests that the availability of salmon on both sides of the Atlantic is affected by common environmental conditions.

Catch records of this kind stimulate speculations on future catches; however, it is impossible to make predictions about Atlantic salmon catches with the degree of accuracy that is possible for many other kinds of fish. Quite good information can be obtained on the number of young salmon produced in rivers each year, up to the time when they run to sea as smolts. But it is hard to estimate the number that will survive and be caught as adults. Salmon live at unknown places in the sea for a variable number of years. A large proportion of them, possibly 95%, disappear for unknown reasons. Adding to the difficulties are uncertainties of the fishing operations themselves, which are so often handicapped by unfavourable weather and water conditions.

Reproduced from *Atlantic Salmon Journal*, No. 3, pp. 5–6, 1960. FRB No. 632



By 1958, research by the Fisheries Research Board of Canada had given much information on the effects on young salmon production of several years of DDT spraying to control an outbreak of spruce budworms in New Brunswick forests. The facts were used as a basis for predicting "below normal" stocks of adult salmon within a few years (April 1958 issue of *The Atlantic Advocate*, pages 65-68). Care was taken to state that "normal" meant the total annual catch, whatever it might be, without any effects of DDT spraying. The predictions that marked reductions would occur in adult stocks returning to affected areas still stand. Unusually low runs of grilse in the early summer of 1960 into areas sprayed heavily in 1956 and 1957 appear to confirm the predictions. It is expected that the adverse effects of DDT spraying will be largely temporary, unless it is continued on the same areas year after year.

There may be continuing serious pollution of salmon rearing areas by application of pesticides, or other harmful changes in the river environment of young salmon associated with industrial developments. If such handicaps do not become too serious, it seems reasonable to assume that commercial catches will again return to at least the past average level of 7 million pounds per year. Still further improvement is possible through management procedures that are now being investigated, such as predatory bird control and the planting of hatchery-reared stock of smolt size.

This feeling of moderate optimism is strengthened by the recent return to normal of the average annual water temperature off our coast, after being two to three degrees Fahrenheit above normal during the period 1949-1954. There has been an inverse relationship between sea temperatures and commercial salmon catches since 1921 when the temperature records were started. That is, the years with highest average water temperatures usually had the lowest salmon catches. In the years around 1925 and 1930 when peak catches were made, sea temperatures were either normal or below normal. This may be only a coincidence without any biological significance. It is possible, though, that increased water temperature might affect the habits of salmon in the sea and cause them to avoid netting areas. Or higher water temperature might cause lower smolt survival in the sea. It is expected that sea temperatures will continue at normal or sub-normal levels over the next few years.

PROTEINS IN FISH MUSCLE

16. ADENOSINETRIPHOSPHATASE ACTIVITY OF COD MYOSIN AND ACTOMYOSIN[1]

J. R. Dingle and J. A. Hines

Abstract

The adenosinetriphosphatase (ATPase) activities of actomyosin extracts of prerigor and postrigor cod muscle, and of myosins prepared from them by ultracentrifugation in the presence of ATP, have been measured at ionic concentration 0.1 and pH 7.3. The actomyosin ATPases were strongly activated by Mg^{++}, while those of the myosins were suppressed by Mg^{++}, in agreement with corresponding rabbit skeletal muscle proteins. Ca^{++} ion also moderately activated the actomyosin ATPases, but had little effect on the myosin ATPases. When actomyosin was precipitated by dilution in the presence of ATP and Mg^{++}, the characteristic activation by Mg^{++} was lost. The myosin ATPases were very labile.

Procedures for the direct extraction of myosin from mammalian tissue, such as the classical method of Szent-Györgyi (1) and its subsequent modifications, have not so far been successfully applied to fish muscle. A method due to A. G. Szent-Györgyi (2), based on the irreversible dissociation of actomyosin by potassium iodide, yielded a protein from cod muscle that appeared to be a modified myosin (3). Connell (4) reported the preparation of cod myosin by ultracentrifugation of a mixture of actomyosin and adenosinetriphosphate (ATP), following a method first used by Weber (5) for rabbit myosin. The sedimentation and diffusion characteristics of Connell's preparation indicated that it was analogous to mammalian myosin. We have confirmed the success of this procedure, but further evidence of this analogy could be useful. We have investigated the ATPase activity of such preparations, and also that of so-called actomyosin extracts of both prerigor and postrigor cod muscle.

It has been known for some time that both actomyosin and myosin from mammalian sources, principally rabbit, possess adenosinetriphosphatase activity (6). The enzymic activities of the two proteins differ, however, in the presence of magnesium or calcium ions at low ionic concentration ($\Gamma/2$) (7, 8, 9). For example, at $\Gamma/2 = 0.10$ or less, and pH 7.0, 10^{-3} M $MgCl_2$ activates actomyosin ATPase, but inhibits myosin ATPase. This difference in response to activators was used by Weber (5) to help identify her preparation of myosin. The results of the present investigation confirm that the material prepared by the ultracentrifugal procedure is analogous to mammalian myosin. Because of the great lability of cod myosin, already mentioned by Connell (4), it has not so far been feasible to carry out a thorough kinetic study of the enzyme reaction, and the present results must be regarded as hardly more than semiquantitative.

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Materials

Fillets cut from cod (*Gadus callarias*), obtained from the local inshore fishery, were used in the preparation of the protein extracts. The fish, which were of small to medium size (15 to 25 in.), were transported alive in small tanks to the laboratory, and unless used immediately, were held in a tank at approximately 2° C. The fish were either stunned, or anaesthetized with tricaine methanesulphonate (Sandoz Pharmaceuticals, MS 222), and filleted at once. Portions of fillets not used for immediate extractions were stored in covered beakers at 1° C. Extractions could thus be made on aliquots of the same fillet both before and after it had passed through rigor.

Adenosine-5′-triphosphate was obtained as the crystalline disodium salt from Schwarz Bioresearch Inc. Tris(hydroxymethyl)aminomethane ("Tris", Nutritional Biochemicals Inc.) was twice recrystallized from methanol (10) but the purification had no significant effect on the enzyme reaction, or on the stability of the myosin. The other materials, which were all of reagent grade, were used without further purification.

Methods

Samples cut from the middle of the fillet, and weighing 22.5 g, were extracted in a Waring blendor, fitted with a baffle plate (11) and operated at 90–100 volts. Two extraction procedures were used.

Procedure A

The sarcoplasmic constituents were removed by two 1-minute blendings with 0.05 *M* histidine, pH 6.6, using 425 ml and 375 ml of solution successively. This was followed by a 2-minute extraction of the fibrillar proteins from the residue with 375 ml of KCl–Tris solution (0.55 *M* KCl, 0.05 *M* Tris, pH 7.3). After each blending, the mixture was centrifuged at $1300 \times g$.

Procedure B

A total soluble protein extract was made by blending the sample for 2 minutes with 425 ml of the above KCl–Tris solution. In order to eliminate the water-soluble proteins, the extract was diluted 10-fold with water at 20° C, centrifuged at once for 8 minutes at $1300 \times g$, and the precipitated fibrillar proteins taken up in 3.0 *M* KCl–Tris, pH 7.3, to a final ionic concentration of 0.6. This process was repeated once.

Except for the use of water at 20° C in the precipitation steps, all operations were carried out in a cold room at 2° C, using precooled apparatus. The extracting solutions were degassed by suction, and partly frozen (about 5%) in order to reduce the temperature rise during blending. The use of water at 20° C for dilution greatly increased the speed of precipitation of the protein, and decreased the degree of centrifugation required. In this way, irreversible interactions between the precipitated proteins appear to be held to a minimum since there is little difference in the sedimentation patterns before and after precipitation (12).

In the prerigor extractions by procedure B, the final residue remaining undissolved was rather voluminous. The residue from procedure A was

usually similar, but in two cases it was very small. The residues from both types of postrigor extraction were always very small. The final yields of "actomyosin" were about 40% of the total protein N of the muscle in prerigor extractions, and in postrigor extractions about 65% by procedure A, and 50% by procedure B. The latter figure may be low because of incomplete precipitation. Allowing about 3% of total protein N in cod muscle for stroma (11) and 22% for water-soluble proteins (13), the maximum yield of salt-soluble ("actomyosin") proteins should be about 75% of the total muscle protein N.

After centrifuging at 2000×*g* to remove debris, 200 ml of the final extract or solution was mixed with 20 ml of an ATP–$MgCl_2$ solution (0.055 *M* ATP, 0.055 *M* $MgCl_2$, 0.55 *M* KCl, 0.05 *M* Tris, pH 7.3). The mixture was centrifuged at once for 1 hour at 42,040 r.p.m. in a Spinco model E ultracentrifuge, using a preparative rotor cooled to approximately 0° C. The supernate, which was usually water-clear, and presumably contained myosin, was easily decanted from the residue.

The myosin preparation was dialyzed in Visking cellulose tubing against two changes of 5 volumes of the KCl–Tris solution, $\Gamma/2 = 0.60$, pH 7.3, at about 2° C for a total of 24 hours. Similar dialysis of the untreated actomyosin extracts did not alter the enzymic characteristics, although the phosphate blank was decreased. This step was therefore usually omitted for actomyosin in order to make the enzyme assays within a few hours of preparation.

Reagents used in the enzyme reaction were dissolved in water and the pH adjusted to 7.0–7.5. The reaction mixture contained 0.05 *M* Tris buffer, pH 7.3, and the ionic concentration was adjusted where required by addition of KCl or NaCl. Other "standard" concentrations were: ATP, 0.0015 *M*; $MgCl_2$, 0.0015 *M*; $CaCl_2$, 0.01 *M*; these were found in separate experiments to yield maximum ATPase activity in actomyosin preparations at $\Gamma/2 = 0.1$. The protein concentration in the reaction mixtures varied from 0.02 to 0.05 mg N/ml. A mixture of appropriate amounts of the above reagents was made up to 25 ml. An aliquot of protein solution (2.5 ml) was diluted to 25 ml with water of the desired reaction temperature, and the resulting protein suspension added at once to the substrate mixture to start the reaction. It was found that if an aliquot of protein stock was added directly to an appropriate volume of substrate mixture, so that the dilution of the protein occurred in the presence of the ATP, the characteristic activation of actomyosin ATPase by Mg^{++} at low ionic concentration was lost.

The enzyme assays were carried out in a constant temperature water bath, stirring the mixtures with a submerged magnetic stirrer enclosed in a plastic bag. Aliquots of 1 ml were withdrawn at appropriate intervals, mixed rapidly with 9 ml of 10% trichloracetic acid, the precipitated protein removed by centrifuging, and 5 ml of the supernate analyzed for inorganic phosphate by the method of Rockstein and Herron (14). In separate experiments, the rate of non-enzymic hydrolysis of ATP in the assay procedure was found to be 0.07% per minute by the trichloracetic acid, and 0.09% per minute by the acid molybdate mixture. This was considered to be negligible for the purpose of this work.

Protein concentrations in the stock solutions were estimated by the biuret procedure of Snow (15). Interference from the Tris buffer was reduced to negligible levels by including it in the blank.

Sedimentation Analysis

Sedimentation analyses were made in the Spinco model E ultracentrifuge at 59,780 r.p.m. The rotor was precooled, and its temperature was maintained at approximately 0° C. The sedimentation constants (s') are given in Svedberg units for the conditions of the run, and are not corrected to water at 20° C.

Patterns found for the final "actomyosin" preparations by procedures A and B from the same sample of cod muscle both before and after it had passed through rigor mortis are shown in Figs. 1*a* and 1*b*. The total areas of the procedure A patterns were somewhat smaller than those of the procedure B patterns, even though the protein concentrations were adjusted to be equal; this may be due in part at least to a larger amount of rapidly sedimenting gel in the A preparations. It is also interesting that in the patterns of the prerigor B preparation (Fig. 1*a*, bottom) the amount of component III appeared to be unusually large relative to component II. The patterns resemble those reported by Ellis and Winchester (12) except that the peak due to their component I was now less prominent. It was found, however, that dialysis of an extract made by their procedure against the KCl–Tris system used in the present work led to a considerable decrease in the size of peak I.

The sedimentation patterns of the myosins prepared from the extracts just described are shown in Figs. 2*a* and 2*b*. Again, the total areas in the preparations derived from the method B extracts were somewhat larger than those from the A extracts, even though the total protein concentrations were equal. Each preparation had a small amount of rapidly sedimenting material, another component sedimenting just ahead of the main one, and a smaller amount of component IV. Some myosin preparations, however, appeared to be monodisperse. Examples made from both A and B extracts of a postrigor cod from a commercial source are shown in Fig. 2*c*. Only a trace of a rapid component could be discerned at an early stage of this run. The reasons for these differences are not known.

The sedimentation constant of the main peak in the myosin preparations did not appear to depend on whether NaCl–phosphate, used by Ellis and Winchester (12), or KCl–Tris was used as the solvent, but it did depend moderately on the protein concentration. Available data on the latter property are plotted in Fig. 3. The constant agreed well with that of component III ($s' = 3.1$–3.7) of the untreated "actomyosin" extracts. After an approximate correction to a liquid of the viscosity and density of water at 20° C, the sedimentation constant of the myosin at zero concentration became 6.8, in fair agreement with the value 6.43 found by Connell (4) and also with that of component III in the extracts of Ellis and Winchester (12).

Tests for Water-soluble ATPase

Kielley and Meyerhof (16) reported the preparation from rat muscle of a

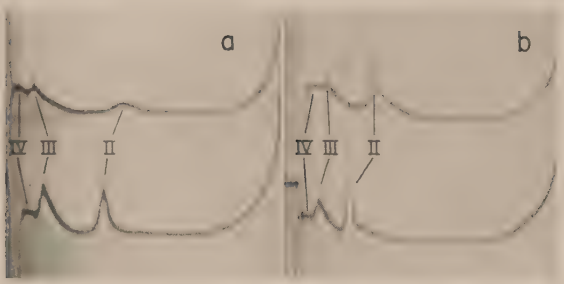

FIG. 1. Sedimentation patterns of actomyosin extracts of cod muscle. (*a*) Prerigor, 0.51 mg N/ml. (*b*) Postrigor, 0.69 mg N/ml. Upper trace, extract by procedure A. Lower trace, extract by procedure B. After 24 minutes at 59,780 r.p.m., $T \sim 0°$ C. Solvent, KCl–Tris, $\Gamma/2 = 0.60$, pH 7.3. Numbering follows Ellis and Winchester (12). Sedimentation to the right.

FIG. 2. Sedimentation patterns of myosin preparations from cod muscle. (*a*) Prerigor, 0.40 mg N/ml. (*b*) Postrigor, 0.47 mg N/ml. Upper trace, myosin from extract by procedure A. Lower trace, myosin from extract by procedure B. After 160 minutes at 59,780 r.p.m., $T \sim 0°$ C. Solvent, KCl–Tris, $\Gamma/2 = 0.60$, pH 7.3. (*c*) Postrigor. Upper trace, from extract by procedure A, 0.45 mg N/ml. Lower trace, from extract by procedure B, 0.38 mg N/ml. After 144 minutes at 59,780 r.p.m. Other conditions as in (*a*) and (*b*). Sedimentation to the right.

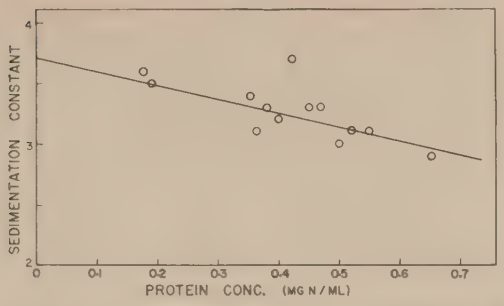

FIG. 3. Dependence of sedimentation constant of ultracentrifuge myosin on protein concentration. Constants are for the conditions of the run: $T \sim 0°$ C; 0.55 *M* KCl+0.05 *M* Tris, pH 7.3, or NaCl/phosphate, $\Sigma \Gamma/2 = 0.60$, pH 7.0.

Mg-activated ATPase, which was not myosin or actomyosin, but was associated with particulate matter remaining suspended after dilution of the preparation to low ionic concentration. To test for possible interference from a similar enzyme in our extracts, ATPase assays were made upon the following at $\Gamma/2 = 0.1$, in the presence of 0.0015 *M* $MgCl_2$:

(*a*) an extract of muscle made with histidine buffer, $\Gamma/2 = 0.05$, pH 6.6;

(*b*) the supernate remaining after precipitating protein, by a 10-fold dilution with water, from a KCl–Tris extract ($\Gamma/2 = 0.60$, pH 7.3) of the residue from extraction (*a*);

(*c*) the supernate remaining after two precipitations, by 10-fold dilution, of protein from a KCl–Tris ($\Gamma/2 = 0.60$, pH 7.3) extract of muscle. In each of these procedures, insoluble material was removed by centrifuging at $1300 \times g$. The samples were assayed without further dilution, but in no case was any activity detected. It may be that the white muscle of cod and other species is very poor in the Kielley–Meyerhof enzyme; Tonzetich and Kare (17) have reported that they could prepare a water-soluble ATPase from the red muscle of hen, but not from the white muscle.

ATPase Activities

The ATPase reaction curves for the actomyosin preparations whose sedimentation patterns have already been described are shown in Figs. 4*a* and 4*b*. The experimental points were corrected for phosphate blank (see below) before plotting. The shapes of the curves and the relative reaction rates calculated from them were similar for a number of other preparations studied, although the absolute rates varied from one extract to another.

After 5 minutes of reaction at 20° C, the amount of inorganic phosphate produced in the most active reaction cited in Fig. 4 corresponded to the hydrolysis of 65% of the ATP originally present. Even when this change in substrate concentration was allowed for, in this and other preparations, a linear relation for rate of hydrolysis could not always be demonstrated. It is not unlikely that some denaturation of the enzyme occurred during the reaction at 20° C or higher, but this was not fully investigated.

It was convenient to extrapolate the reaction curves to zero time to estimate the phosphate blank. This would not reveal the presence of any initial burst

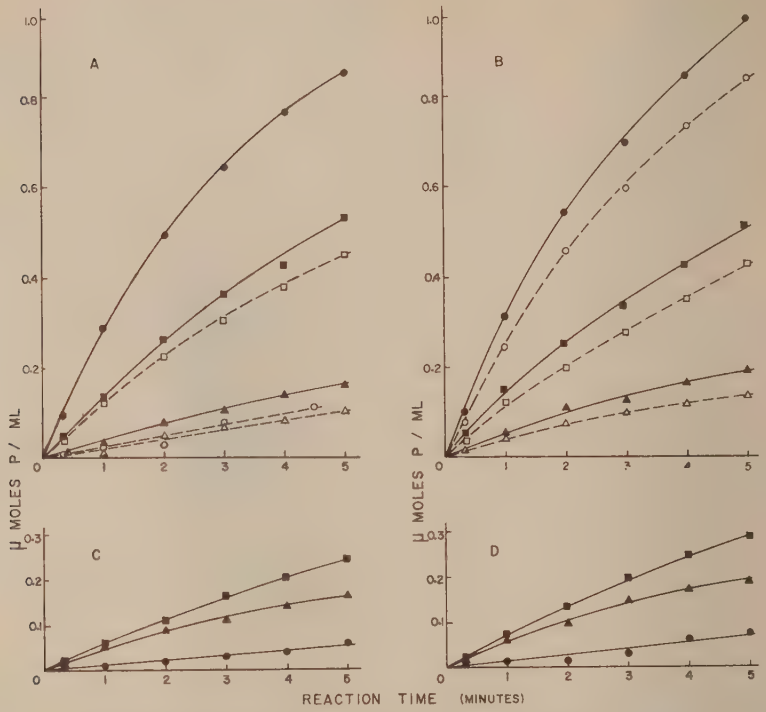

FIG. 4. Reaction curves of cod actomyosin and myosin ATPases. $T = 20°$ C; I/2∼0.1; pH 7.3. (A) Actomyosin, prerigor. (B) Actomyosin, postrigor. (C) Myosin, prerigor. (D) Myosin, postrigor. (In C and D, curves for myosin from procedure B extracts omitted for clarity.)

● ○ Activated by Mg^{++}, 0.0015 *M*. ■ □ Activated by Ca^{++}, 0.01 *M*. ▲ △ No activator. Full curves, extracts by procedure A. Broken curves, extracts by procedure B.

of reaction such as has been reported for actomyosin in 0.6 *M* KCl by Tonomura and Kitagawa (18). Direct checks of phosphate blanks in several runs agreed quite closely, however, with those found by extrapolation. The reaction rates were calculated from the amount of hydrolysis that occurred in the first 60 seconds of reaction as indicated by a smooth curve drawn through the experimental points.

The reaction curves found for the myosins (Figs. 4*c* and 4*d*) prepared from the above actomyosin extracts were similar in shape to those found for the actomyosins.

The rate constants calculated for all the reactions carried out in this representative experiment (No. 37) are given in Table I. The ATPase activities of the actomyosin extracts from postrigor muscle, prepared by both methods, and of that made by method A from prerigor muscle, were all activated by Ca^{++}, but were more strongly activated by Mg^{++}. On the other hand, that made by method B was activated by Ca^{++}, but not at all by Mg^{++}. All of the myosin ATPases were rather insensitive to Ca^{++}, and strongly inhibited by Mg^{++}. Moreover, the myosins were about twice as active as the actomy-

TABLE I

ATPase activities of actomyosin extracts and of ultracentrifuge myosin from prerigor and postrigor cod muscle ($\Gamma/2 \sim 0.10$; pH 7.3 (Tris); $[Mg^{++}] = 1.5 \times 10^{-3}$ *M*; $[Ca^{++}] = 10^{-2}$ *M*; $[ATP] = 1.5 \times 10^{-3}$ *M*. Activities in μmoles P/mg N/min)

Experiment No.	Extraction procedure	Activator					
		Mg^{++}	Ca^{++}	Nil	Mg^{++}	Ca^{++}	Nil
		Actomyosin			Myosin		
37 ($T = 20°$ C)	Prerigor, A	6.9	3.3	0.86	0.38	2.4	2.2
	Prerigor, B	1.0	4.9	0.90	0.78	1.8	2.4
	Postrigor, A	6.8	3.5	1.2	0.50	2.6	2.1
	Postrigor, B	7.2	3.5	1.2	0.44	3.0	2.1
35 ($T = 20°$ C)	Prerigor, A	8.0	3.6	0.91	—	—	—
	Prerigor, B	1.0	4.8	1.0	—	—	—
	Postrigor, A	8.4	4.0	0.95	—	—	—
	Postrigor, B	12.0	3.9	1.1	—	—	—
8 ($T \sim 13°$ C)	Prerigor, A	8.2	3.5	1.8	0	2.3	2.3
	Postrigor, A	7.3	3.3	1.3	0.1?	2.1	2.8

osins when no bivalent ion was added, a difference that seems too great to be accounted for by the presumed loss of actin from the myosin preparations.

Other data in Table I are included to illustrate reproducibility of activities. Not every preparation of myosin was active, but those that were active were comparable. The activities of actomyosin in the presence of Ca^{++} were reasonably constant, but those in the presence of Mg^{++} were rather variable. The latter were always greater than the Ca^{++} activities, however, except in the case of prerigor preparations by method B, which were always lower than the corresponding Ca^{++} activities.

In Table II are given some ATPase activities for actomyosin and myosin reported by others. Nearly all of the preparations were made from prerigor rabbit muscle, and the conditions of assay approximated those used in the present work.

It was possible to obtain identical reaction curves with the actomyosin preparations in duplicate assays made within an hour of one another. Runs made on successive days, however, showed a decreasing activity. The activity of the myosin preparations was even more labile; within 24 hours after dialysis (48 hours after preparation) the activity had usually fallen to zero.

In one experiment, samples of frozen cod that had been stored at −23° C for 13 days and for 7 months were extracted with 0.6 *M* KCl-Tris, pH 7.3. The yields of total extractable protein were 78% and 79% of the total protein N of the muscle, respectively, so that only a small amount of protein had become non-dispersible. A water extract of the 13-day sample gave a small activity (1.50 μmoles P mg N min) in the presence of Mg^{++}, which may have been due to a Kielley-Meyerhof enzyme. The "total" extracts of both samples exhibited strong activities of the actomyosin type, being strongly activated by Mg^{++} (Table III). It appears then that while the ATPase activities of the muscle proteins are labile once the proteins have been extracted, they are relatively very stable when left *in situ* in the frozen state. A similar stability

TABLE II

Representative ATPase activities of actomyosin and myosin from prerigor rabbit muscle, at $\Gamma/2 \sim 0.1$
(Activities given as μmoles P/mg N/min)

Reference	Activator						Conditions
	Mg^{++}	Ca^{++}	Nil	Mg^{++}	Ca^{++}	Nil	
	Actomyosin			Myosin			
25	1.08	3.3	0.60	—	—	—	0.04 *M* KCl } Tris, pH 7.5,
	0.39	3.0	0.77	—	—	—	0.08–0.10 *M* KCl } *T* = ?
8	1.44	2.1	1.14	0.60	1.80	0.18	0.07–0.10 *M* KCl; glycine, pH 7.0, *T* = 20° C
9	2.2	3.6	1.5	—	—	—	0.10 *M* KCl; 0.01 *M* histidine, pH 7.0, *T* = 21° C
5	1.3	2.6	—	0.07	2.7	—	0.06–0.08 *M* KCl; histidine, pH 6.6–6.9, *T* = 15–20° C
26	1.8	2.5	—	1.1	3.4	—	$\Gamma/2 = 0.08$; histidine, pH 6.7, *T* = 25° C
27	—	—	—	—	2.1 to 5.1	—	*T* = 25° C } $\Gamma/2 = 0.19$; 0.05 *M*
	—	—	—	—	0.39	—	*T* = 0° C } Tris, pH 7.2
28	—	—	—	—	3.3	—	0.004 *M* $CaCl_2$ } no buffer, pH 7.3,
					4.7		0.04 *M* $CaCl_2$ } 0.15 *M* KCl, *T* = 27.5° C
29	—	—	—	—	7.1	—	0.05 *M* KCl; 0.005 *M* $CaCl_2$; 0.02 *M* histidine, pH 7.6, *T* = ?

TABLE III

ATPase activities of actomyosin extracts from frozen cod muscle
(Activities given as μmoles P/mg N/min. Fish frozen postrigor)

Storage	Activator		
	Mg^{++}	Ca^{++}	
13 days at −23° C	10.8	3.2	Total extract, not precipitated
	10.5	3.0	Extract precipitated once
7 months at −23° C	—	3.2	Total extract, not precipitated
	8.0	1.8	Extract precipitated once

has been noted for the ATPase of frozen fish by Partmann (19) and Connell (20).

In another experiment, KI–myosin (3) was prepared from postrigor cod muscle, and a sample of ultracentrifuge myosin was prepared from the same fillet. The latter preparation exhibited normal ATPase activity, but the KI–myosin had no activity in the presence of 10^{-2} *M* Ca^{++}. Moreover, when the ultracentrifuge myosin was treated with 0.6 *M* KI, which was subsequently removed by dialysis, its ATPase activity was abolished. After addition of the KI, and before dialysis, the total ionic concentration was 1.2, but it is known from other work that similar treatment with KCl does not materially alter the ATPase activity of ultracentrifuge myosin. It appears, therefore, that if the protein prepared by treatment of actomyosin with KI is myosin, as claimed by Szent-Györgyi (2), then it must be a denatured form of that protein.

Discussion

A comparison of the representative data presented in Table I suggests that, as far as ATPase activity is concerned, ultracentrifugally prepared cod myosin is analogous with mammalian myosin prepared by the usual extractive methods. Not only was the characteristic inactivation by Mg^{++} at low total ionic concentration observed, but the magnitudes of the activities under various conditions agreed fairly well with those of the mammalian myosins. The results of the sedimentation analyses also tended to confirm the analogy. While in some of our preparations the heterogeneity suggested that the dissociation of the actomyosin had been incomplete, or that some aggregation of the myosin had occurred, the estimated sedimentation constant of the main component, $s_{20,w} = 6.8 \times 10^{-13}$, agreed with that of rabbit myosin (6.7–7.2) (21).

The case of the actomyosin extracts is rather less clear. Extracts of fish muscle made at moderate ionic concentrations, with or without prior removal of the sarcoplasmic constituents, consistently showed complex patterns in the ultracentrifuge. The very similar sedimentation behaviors of component III and of myosin strongly suggests that they are identical. If this is so, then the ATPase activities observed at low ionic concentration for "actomyosin" preparations may be the resultant of the activities of myosin and of true actomyosin(s) (presumably peaks I and II) (12). On the other hand, it is possible that lowering the ionic concentration to approximately

0.1 increases the proportion of actomyosin at the expense of myosin. Except for the work of Johnson and Landolt (22) and of Ellis and Winchester (12), the complex nature of actomyosin extracts has not usually been considered in studies of muscle proteins.

The low value for ATPase activity, in the presence of Mg^{++}, of prerigor actomyosin preparations made by procedure B was reproducible. Moreover, if any actomyosin extract, prerigor or postrigor, by method A or B, was added directly to the substrate mixture containing ATP and Mg^{++}, similarly low activities were consistently found. The common factor appeared to be precipitation of actomyosin in the presence of ATP and Mg^{++}. It is tempting to speculate that under these conditions the precipitated protein was largely myosin and that the characteristic suppression of myosin ATPase was being observed. This would follow from the dissociation of actomyosin that almost certainly occurs in the presence of ATP at $\Gamma/2 = 0.5$–0.6. On the other hand, this would not account for the other aberrations in the activities of actomyosin and myosin made from prerigor muscle by procedure B, apparent in Table I, and which appear to be reproducible from extract to extract. Nor was this hypothesis supported by an experiment in which an actomyosin extract of postrigor cod was mixed with ATP and $MgCl_2$ and then precipitated by dilution with water. Not all of the precipitated protein would redissolve in 0.6 *M* KCl but the ATPase activity of that portion which did dissolve was still enhanced by Mg^{++}.

In connection with these phenomena, the results of Saito and Hidaka (30) are interesting. Working with prerigor muscle, these authors claim to have prepared myosin A and myosin B by 20-minute and 24-hour extractions respectively, using a Greenstein-Edsall type solution. Both preparations were then precipitated several times to purify them. With both types of extract, the ATPase activity was enhanced about fourfold by Ca^{++} and reduced to about 50% by Mg^{++}. This behavior is similar to that of our prerigor "actomyosin" extract, using procedure B, rather than that of ultracentrifuge myosin. On the other hand, it is not clear from Saito and Hidaka's paper whether the reaction was carried out in 0.25 or 0.025 *M* KCl. The former appears to be the more likely, and if so, their results cannot be compared with ours because the influence of ionic strength on the reaction is known to be important in this range.

Saito and Hidaka (31) also report that while the activity of their myosin A and B fractions was rapidly reduced at elevated temperatures (37–45° C), only small deterioration was observed after storage for 48 hours at 1–3° C. This also agrees wth our experience with actomyosin preparations rather than with ultracentrifuge myosin.

It is usually stated that actomyosin is characteristically "strongly" activated by Mg^{++} at low ionic concentration (24). While this does seem to occur with cod actomyosin, it appears to be much less marked with mammalian actomyosin. It may be significant that the latter was usually prepared from prerigor muscle by a procedure analogous to our method B.

The original object of this work required that the ATPase activities be

studied at low ionic concentration, so that the system was necessarily heterogeneous. Because of this, the complex nature of the actomyosin extracts, and the extreme lability of the myosin, it did not seem worthwhile to carry out a thorough kinetic study at present. The effects of pH, temperature, and ionic strength will be presented in a later report, and a study of the ATPase activities of the fibrils is also being undertaken.

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NUTRITION AND METABOLISM OF MARINE BACTERIA

X. THE GLYOXYLATE CYCLE IN A MARINE BACTERIUM[1]

R. A. MacLeod,[2] Aiko Hori,[3] and Sylvia M. Fox

Abstract

Extracts of a species of marine bacterium have been shown to contain isocitratase and malate synthetase when cells were grown in a medium in which acetate was the sole source of carbon and energy. Neither enzyme could be demonstrated in extracts prepared from cells grown in a nutrient broth, yeast extract medium.

Isocitratase, which converts isocitrate to glyoxylate and succinate (11), and malate synthetase, which condenses acetyl coenzyme A and glyoxylate to form malate (14), together make up what has been termed the glyoxylate bypass in the tricarboxylic acid cycle (4). The operation of this bypass, serving as a source of the four-carbon compounds necessary for the continued operation of the tricarboxylic acid cycle, provides an explanation for the capacity of cells to grow in a medium containing acetate as the sole source of carbon and energy.

Both isocitratase and malate synthetase have been shown to be present in a wide variety of microorganisms (6). In view of the possibility that terrestrial bacteria have evolved from marine bacteria (7) it was of interest to know if these enzymes are also present in a bacterium of marine origin. In this report a species of marine bacteria which has been found to contain all of the enzymes of the tricarboxylic acid cycle (9) is shown also to be capable of forming the key enzymes of the glyoxylate bypass.

Experimental

The marine bacterium used in this study is a species of either a *Pseudomonas* or a *Spirillum* and has been designated B-16 in this and other communications. The organism was isolated from marine clams and has been shown to have a highly specific requirement for Na^+ for growth. Its marine origin coupled with its need for Na^+ serves to distinguish this bacterium from terrestrial species (7).

Two growth media were used in this study. One, referred to as the acetate medium, contained the following (in grams per liter): sodium acetate, 20; NaCl, 12.7; $MgCl_2.6H_2O$, 5.32; $(NH_4)_2SO_4$, 1.0; KCl, 0.73; K_2HPO_4, 0.105; KH_2PO_4, 0.105; and $FeSO_4(NH_4)_2SO_4.6H_2O$, 0.035. The other contained nutrient broth 8, and yeast extract 5, in place of the sodium acetate.

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F.R.B. No. 634

The methods used to cultivate and harvest the cells and to prepare sonic extracts have been described (8). Growth on the acetate medium was slower than on the nutrient broth, yeast extract medium. With the former, 40–45 hours', and with the latter, 18 hours' incubation were required to obtain a good yield of cells.

Except where indicated, cells were washed and suspended in 0.02 *M* phosphate buffer containing 0.5 mg per ml of glutathione.

Isocitratase activity was measured in an assay system which was essentially that of Smith and Gunsalus (13) modified to provide conditions more nearly optimum for the enzyme from this organism. The assay was carried out in 15-ml Warburg flasks attached to the manometers of a Warburg respirometer. Each flask contained Tris[4] buffer pH 7.5, 125 μmoles, DL-isocitrate (Tris salt) 40 μmoles, $MgCl_2$ 6 μmoles, cysteine 4 μmoles, and sonic extract of the cells. The total volume was 3 ml, the gas phase N_2, the temperature 25°, and the incubation time 60 minutes (unless otherwise indicated). The reaction was stopped by addition of 0.2 ml of 80% trichloracetic acid, the mixture was centrifuged at 50,000 *g* for 30 minutes to remove protein and the supernatant analyzed for glyoxylate formed. The results reported were corrected for glyoxylate formation in flasks identical except for the addition of substrate to the reaction mixture after the reaction had been stopped by trichloracetic acid.

Glyoxylate was measured as the 2,4-dinitrophenylhydrazone according to the procedure of Smith and Gunsalus (13) using glyoxylic acid (California Foundation) as the standard. Although the latter was a "purified" rather than a "chemically pure" reagent, it gave a linear relation between optical density and concentration in the colorimetric test applied and hence was considered satisfactory for the purposes intended. Glyoxylate was identified on paper chromatograms as its 2,4-dinitrophenylhydrazone using the ammonia, butanol solvent system of Cavallini *et al.* (1). Reversal of the isocitratase reaction was shown by demonstrating isocitrate formation from glyoxylate and succinate. Isocitrate was detected chromatographically using two solvent systems: *n*-butanol, isobutanol, formic acid, H_2O and isoamyl alcohol, formic acid, H_2O (3) as well as spectrophotometrically by following TPN reduction at 340 mμ under conditions optimum for the activity of the isocitric dehydrogenase present in the extract (9, 10).

Malate synthetase was detected by demonstrating malate formation from acetyl CoA and glyoxylate in the presence of cell-free extracts of the organism. Acetyl CoA was generated from acetyl PO_4 and CoA by the action of phosphotransacetylase (Worthington Biochemical Corp.). Malate was demonstrated chromatographically on paper using the solvent system *n*-butanol, isobutanol, formic acid, H_2O (3) and spectrophotometrically by following TPN reduction at 340 mμ under conditions known to be optimum for the malic dehydrogenase present in the extracts (7).

[4]Abbreviations used: Tris, tris(hydroxymethyl)aminomethane; TPN, triphosphopyridine nucleotide; CoA, coenzyme A; ATP, adenosine triphosphate.

Results and Discussion

Demonstration of the Presence of Isocitratase

No evidence of the presence of isocitratase could be obtained in extracts of cells grown in a nutrient broth yeast extract medium. When the cells were grown in a medium containing acetate as the sole source of carbon, extracts prepared from the cells contained the enzyme. This is in accord with observations made previously with terrestrial bacteria that the enzyme forms adaptively only under conditions where the net formation of C_4 compounds from acetate is necessary for growth (5, 12).

The enzyme was detected by the capacity of the extract to form glyoxylate from isocitrate. Glyoxylate was identified both by the position of its 2,4-dinitrophenylhydrazone on a paper chromatogram and by the characteristic red-brown color which developed at the spot on spraying with alkali. The 2,4-dinitrophenylhydrazone of another keto acid, pyruvate, was also extracted from the reaction mixtures, both in the presence and absence of isocitrate. Since this compound could interfere with the determination of glyoxylate by the method used, it was of interest to know if the pyruvate level varied in the reaction mixture upon the addition of substrate. When the optical density readings at 490 and 540 mμ of a system producing glyoxylate from isocitrate were corrected for endogenous color formation, the ratio of corrected optical densities was 2.03. The 490/540 ratio of the 2,4-dinitrophenylhydrazone of glyoxylate is 1.9, in good agreement with this value, whereas those of the 2,4-dinitrophenylhydrazones of pyruvic and α-ketoglutaric acid are 1.1 and 1.0 respectively (13). It would thus appear that the pyruvate level does not vary when the substrate is present and that the color to which it gives rise can be corrected for satisfactorily with the endogenous control.

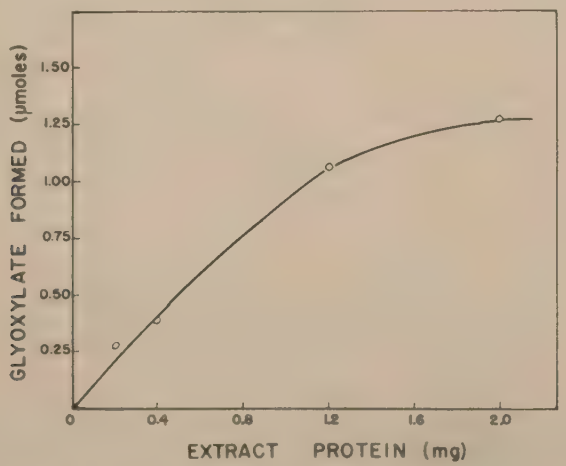

FIG. 1. Glyoxylate formed from isocitrate in response to increasing concentrations of cell-free extract of marine bacterium B-16. Glyoxylate, measured as its 2,4-dinitrophenylhydrazone, was determined after an incubation period of 60 minutes under conditions described in the Experimental section.

Glyoxylate formation from isocitrate in response to increasing concentrations of extract protein is shown in Fig. 1. The response obtained is not linear at higher extract concentrations as might be expected when a crude extract is used and a number of reactions compete both for the substrate and the product formed. Succinate could not be detected in the reaction mixture. Since the reverse reaction, succinate and glyoxylate forming isocitrate, was readily demonstrated, it is reasonable to conclude that succinate must have formed from isocitrate and was further metabolized by the crude extract used.

Reversibility of Isocitratase

Advantage was taken of the fact that the extract of this marine bacterium contains a powerful isocitric dehydrogenase if it is prepared and tested under appropriate conditions (9, 10). Formation of isocitrate from a combination of glyoxylate and succinate was indicated spectrophotometrically by following TPN reduction at 340 mμ under conditions optimum for the activity of the isocitric dehydrogenase in the extract (Fig. 2). Glyoxylate and succinate tested separately were without activity. Malate, which can also cause TPN reduction, was likewise ineffective due to the absence of added K^+, a requirement for the activity of the malic dehydrogenase present in the cells (8). Isocitrate formed from glyoxylate and succinate in this system was identified by paper chromatography.

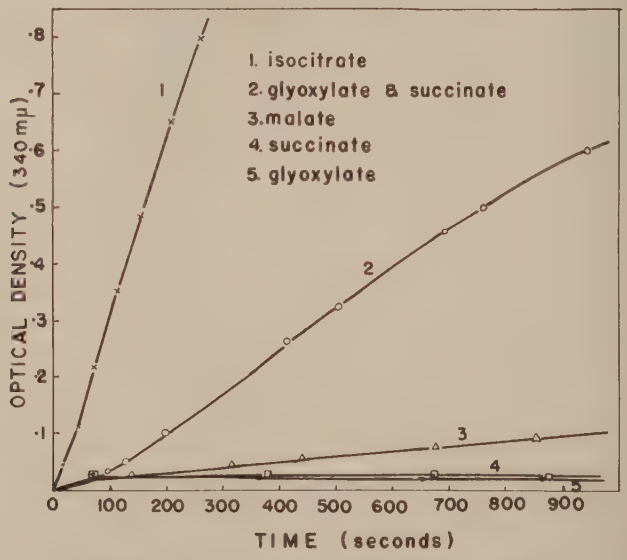

FIG. 2. Spectrophotometric evidence for the reversal of the isocitratase reaction, showing capacity of glyoxylate and succinate together to cause TPN reduction under conditions optimum for the activity of the isocitric dehydrogenase present in the bacterial extract. Complete system: Tris buffer pH 7.5, 250 μmoles; $MgCl_2$, 50 μmoles; TPN, 1 μmole; cell-free extract ≃ 15 mg protein. Substrates added, where indicated, in following amounts: glyoxylate, 10 μmoles; succinate, 20 μmoles; *dl*-isocitrate, 20 μmoles; malate, 20 μmoles. Total volume, 3 ml. Temperature 25°.

Malate Synthetase

Spectrophotometric evidence for the presence of malate synthetase in the marine bacterial extract is presented in Fig. 3. Under conditions giving rise to acetyl CoA, the presence of glyoxylate in a system containing the extract caused TPN reduction after a suitable incubation period when the pyridine nucleotide coenzyme for the malic dehydrogenase of this organism was added to the system. Under the conditions used, a 60-minute incubation period prior to the addition of TPN was the minimum required to cause a reasonable rate of reduction. Neither glyoxylate nor acetyl phosphate alone produced a change in optical density but malate was effective. That malate was indeed the product of the reaction was indicated by paper chromatography of an ether extract of the acidified reaction mixture.

No evidence of malate synthetase activity was obtained in enzyme extracts from cells grown on nutrient broth yeast extract medium. In other microorganisms examined, malate synthetase is much less dependent on growth substrate than isocitratase (6). Although, in this experiment, phosphotransacetylase was used to generate acetyl CoA, an acetate-activating system requiring ATP and CoA can be demonstrated in extracts of acetate-grown cells of this organism (9).

It is clear from these experiments that the key enzymes of the glyoxylate bypass can be demonstrated in this species of marine bacterium under conditions which would be expected to require their presence.

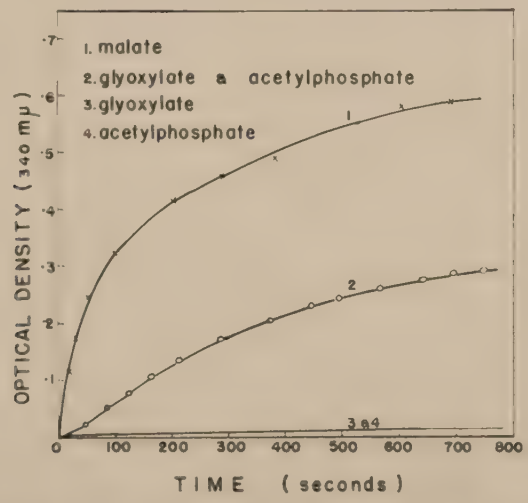

FIG. 3. Spectrophotometric evidence for the presence of malate synthetase in cell-free bacterial extracts of marine bacterium B-16, showing capacity of glyoxylate plus acetyl phosphate to cause TPN reduction under conditions optimum for the malic dehydrogenase present. Complete system: Tris buffer, pH 7.5, 150 μmoles; Tris glyoxylate, 25 μmoles; acetylphosphate, 25 μmoles; CoA, 1.25 μmoles; phosphotransacetylase, 0.2 mg; K_2SO_4, 33 μmoles; $MgCl_2$, 100 μmoles; extract ≃ 2.5 mg protein; Tris malate where indicated, 15 μmoles. Total volume, 2.5 ml. Incubated 1 hour at room temperature in 3-ml cuvettes. Then 0.5 μmoles TPN and extract ≃ 2.5 mg protein added and change in optical density recorded.

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